

NATIONAL
URANIUM
TAILINGS
PROGRAM



Energy, Mines and
Resources Canada

Energie, Mines et
Ressources Canada

Canada

THE POTENTIAL OF GENETIC AND
ADAPTIVE CHANGES TO BACTERIA IN
URANIUM MILL TAILINGS

Serial No. 23317- 61725-01-6Q

Reg. No. 155Q. 23317-6-1725

A Report for:

The National Uranium Tailings Program
Energy, Mines and Resources Canada
Ottawa, Ontario

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October 1986
2302.1

102pp

MICROMEDIA

EXECUTIVE SUMMARY

Review of the literature on different strains of thiobacilli, studied primarily in the laboratory, indicates strain variation in several categories that could potentially influence the process of pyritic uranium tailings acidification and mineral dissolution resulting in radionuclide and metal release. The most important strain characteristics in this context involve either oxidation rates or substrate utilization. Metal resistance characteristics are highly variable and highly mobile within and between species, but are probably of little geochemical consequence except when linked genetically to oxidation capabilities.

A simple two-strain population genetic model was used to consider the evolutionary consequences of new strain introduction to the tailings, and estimate approximate timeframes of adaptive change. New strains may be introduced by long-range transport from other tailings sites, or may arise by mutation. Once present, their establishment or elimination depends on their advantages relative to resident strains, in terms of population carrying capacity (k) and resource overlap (α) with resident strains. The rate of establishment depends on the relative intrinsic growth rates (r) of the competing strains.

Estimated timeframes of adaptive change, using generic model parameter values consistent with available literature on strain variation, suggest that new strains can become rapidly established in tailings populations. Establishment over a 2 to 48 m radius from the point of origin could occur within a year, a short period relative to significant geochemical timeframes (e.g., 2 to 3 years for tailings acidification). Thus, microbial adaptive changes could theoretically influence geochemical evolution of pyritic uranium tailings.

The greatest obstacle to accurate prediction of adaptive shifts in tailings is a paucity of empirical data on bacterial species and strains in uranium tailings environments, strain differences between localities, and in situ rates of growth, genetic exchange and movement. Rates of aerial dispersal and survival during long-range transport are critical to estimation of strain introduction probabilities. Recommendations include field studies designed to obtain such data.

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SECTION 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Introduction and Background

The National Uranium Tailings Program (NUTP) is developing mathematical models to predict future events pertaining to uranium mill tailings, and to quantify and assign uncertainties to these predictions. Data and information on the pathways of release of contaminants from the tailings in the long-term are being obtained for this purpose. Contaminants of primary concern are uranium and thorium series radionuclides, as well as heavy metals. Bacteria-catalyzed oxidation of sulphide minerals, namely pyrite, plays an important part in the geochemistry of uranium mill tailings. Consequently, it is necessary to develop an understanding of the potential long-term effects of genetic and adaptive changes possible for these bacteria, and to estimate their probability, timeframe and effect on tailings geochemistry.

Mathematical models which incorporate uranium tailings, geochemical processes and pyrite oxidation, in particular, have been developed previously (e.g., BEAK, 1983, 1984, 1985a; SENES, 1985). The BEAK (1983) model predicts the long-term human dose equivalent commitment arising from various tailings management options. In this model, constant pyrite oxidation parameters were assumed. The BEAK (1984) and SENES (1985) stochastic models assume random variation in key model parameters around a predetermined central value. The later models predict that the rate of oxygen diffusion into the tailings is the primary factor limiting the rate of pyrite oxidation in the aerobic zone. This, in turn, regulates the pH of the tailings and the rate of dissolution of the various mineral matrices which release radionuclides and metals. Little attention has been given to the possibility of systematic variation in model parameters as a result of genetic change and evolution in the bacteria responsible for pyrite oxidation.

The pyrite oxidation and mineral dissolution rates which are fundamental to our understanding and long-term modelling of contaminant release from uranium tailings are influenced by the metabolic processes of organisms within the microbial community. Each species can be characterized by its own rate parameters and sensitivities to environmental conditions such as pH, temperature and oxygen availability. The net properties of the tailings mass with respect to pyrite oxidation and mineral dissolution are, therefore, the combined result of different microbial characteristics and their

relative densities in the tailings. These net properties may change over the long-term as a result of either:

1. ecological succession within the microbial community, or
2. evolution of individual species within the community, depending on:
 - o genetic variation within the species, and
 - o natural selective forces in the tailings environment.

Ecological succession (replacement of one species or variety by another more successful species or variety) is ultimately dependent on the successful characteristics of each species or variety, as determined by evolutionary events.

In order to evaluate the potential for adaptive shifts within the microbial tailings community, an extensive inventory of known microbial species and genetic variants is required. For each strain identified, optimal conditions and tolerances should be defined. These can be compared to expected conditions within the tailings to estimate natural selection pressures that may favour particular species or strains. In turn, from the metabolic processing capabilities of each strain, the effects of such biological changes on the geochemical tailings environment may be anticipated.

1.2 Study Objectives

The general objectives of the study are as follows:

1. to identify the potential genetic and adaptive changes to bacteria occurring in uranium mill tailings;
2. to estimate the probabilities and timeframes of genetic and adaptive changes; and
3. to relate the effect of these changes to the geochemical evolution of the tailings.

1.3 Methodology

1.3.1 Literature Survey

The first step in the study was to identify and collect the relevant literature on genetic variation and adaptive changes in bacteria indigenous to uranium mill (and other pyritic) tailings, and effects of adaptive changes on optimal conditions for growth and pyrite oxidation. A computerized literature search was conducted first. Once the materials identified in this search were collected, further pertinent literature was identified from their reference lists. Several general texts on bacteria and recent theses on bacteria in pyritic ores or tailings were also consulted.

The computer literature search was conducted through eleven commercial databases:

- | | |
|-----------------------|--------------------------------------|
| o GENETOX | - mutation research |
| o EMIC | - mutation research |
| o BIOSIS | - biological abstracts |
| o NTIS | - U.S. government-sponsored research |
| o CHEM. IND. NOTES | - bioprocessing industry |
| o GEOREF | - geology, geochemistry |
| o POLLUTION ABSTRACTS | - waste management |
| o INIS | - uranium waste management |
| o AGRICOLA | - agricultural research |
| o AQUALINE | - aquatic research |
| o CAS | - chemical abstracts |

The greatest success was achieved through the initial search of BIOSIS listings from 1982 to present. Other databases added a few titles, but the majority were duplications. This is a good sign that the searching had identified a majority of the relevant literature.

The TELEGEN (genetic engineering) abstracting service was searched manually. A further proposed database, DOE ENERGY, was not searched as it is restricted to use in the U.S.A. During the review of literature obtained, additional titles were identified or obtained as appropriate. A recent doctoral thesis on Thiobacillus ferrooxidans (Butler, 1985) and several recent textbooks or symposia proceedings also provided excellent reviews of the literature for comparison to our own.

Key words used during the computer and manual searches included the following, either individually or in combinations:

- o Genera: Thiobacill*
 Thermoplasm*
 Metallogen*
 Sulfolob*
- o Industrial: Uranium
 Leach*
 Min*
- o General: Bacteri*
 Microb*
 Plasmid*
 DNA
- o Modifiers: Strain*
 Variant*
 Genetic*
 Succession*

* Asterisk identifies a suffix to the root word (i.e., bacteri* = bacteria, bacterium, bacterial, etc.).

1.3.2 Critical Review

A number of potential problems were identified early in the review. For example:

- o Almost all of the literature relates to laboratory studies of cultured bacteria under controlled conditions. Care must be taken in relating these controlled conditions to the tailings environment. Culturing for many generations coupled with experimental mutagenesis has resulted in many varieties or species which have not been identified in pyritic tailings;

- o Studies relating to optimizing leaching as a mineral extraction technique have also discussed genera, species and varieties which have never been documented in actual tailings;
- o The genetics of Thiobacillus is not well understood (Brierley, 1982; Strauss et al., 1985). One author (Groudev, 1980) felt that T. ferrooxidans was actually a community of different organisms (varieties, species or even genera);
- o Plasmids are the most rapid vector for dissemination of genetic change but most knowledge about plasmid transmission relative to and from Thiobacillus pertains to plasmids originating in E. coli;
- o Most appropriate population and genetic models have been developed for other genera of bacteria (e.g., E. coli) or higher organisms with sexual reproduction; and
- o Information required for modelling is often found to cover a considerable numerical range for any one parameter.

Thus, close scrutinizing of the literature and critical evaluation was essential to ensure the most appropriate parameters and values were used. Emphasis has been placed, wherever possible, on data pertaining to field studies at uranium tailings sites. In cases where generic parameter values had to be obtained by extrapolation from different species, particular attention was placed on the bacterial phylogeny and genetic transfer mechanisms. Relative to this latter, for instance, Sanderson (1976) suggests that chromosomal genes are subject to transfer barriers reflecting phylogenetic relationships between species, whereas genes carried on plasmids are much more easily transferred between unrelated species. In both cases, genetic transfer barriers (res/mod systems) exist (Roberts, (1976); however, plasmid incompatibility systems (Datta, 1979) are more difficult to infer from bacterial phylogeny. Rates of plasmid mediated genetic transfer cited by Sanderson (1976) are about 10^4 times those reported for chromosomal exchange. Thus, for example, genes predisposing toward tolerance of anoxic conditions would be more apt to be transferred from a facultative anaerobe in tailings to Thiobacillus ferrooxidans or T. thiooxidans if they were plasmid borne. Many resistance traits, such as metal tolerance in Thiobacillus (Torma, 1983; Davidson et al., 1985; DiSpirito et al., 1982), are reportedly borne on plasmids. Plasmid mediation of ferrous ion oxidation has also been suggested (Summer and Silver, 1978), although there is disagreement on this point (Martin et al., 1981).

1.3.3 Identification of Most Important and Probable Genetic and Adaptive Changes

The relative importance of potential genetic and adaptive changes were determined by analysis of the geochemical processes responsible for pyrite oxidation, mineral dissolution and radionuclide release in the tailings. The basic geochemical information is summarized by Smith and Shumate (1970).

The most important changes were identified as those which could affect either:

- o the rate of pyrite oxidation or mineral dissolution directly, or
- o utilization of reaction products, which could accelerate reaction rates indirectly, or
- o the dependence of reaction rates on pH, O₂ or temperature, or
- o bacterial tolerance of environmental conditions such as pH, anoxia or cold.

For example, recent reports of proliferation of thiobacilli at the bottom of uranium waste rock piles where anoxic conditions should prevail (Harries and Ritchie, 1983) are significant since they raise the possibility of an increased environmental range and acceleration of pyrite oxidation in deep tailings.

Determination of the most probable genetic or adaptive changes for indigenous organisms was based on preliminary assessment of:

- o changes observed in indigenous and other organisms;
- o general and specific-locus mutation frequencies; and
- o modes and frequencies of genetic transfer among tailings bacteria and other microbes.

For several characteristics considered important and probable, quantitative estimations of adaptive shift timeframes were undertaken. Quantitative procedures are discussed in Section 1.3.4 and Section 5.0.

1.3.4 Quantitative Estimation of Probabilities and Timeframes for Genetic and Adaptive Changes

Genetic changes can arise either by mutation or genetic transfer. The probability of mutation at any genetic locus is estimated as the observed frequency of spontaneous mutation in growing pure cultures. Specific-locus mutation rates are typically on the order of 10^{-8} mutations/locus/generation. However, higher rates can be induced by mutagenic agents such as ionizing radiation. Potential mutagenesis of bacteria in uranium mill tailings as a result of radioactive material in the tailings is discussed in Section 2.4. Mutational effects are incorporated into a simple population genetic model in Section 5.0.

The probability of genetic traits being transferred from other organisms can be estimated from the relative frequencies of donor and recipient organisms in the tailings, and from experimentally determined transfer rate constants. A simple two-component mass action transfer model, based on Levin *et al.* (1979), is incorporated into the population genetic model of Section 5.0, in order to simulate the adaptive process and estimate timeframes.

Genetic strains were considered to differ either in rates of replication under optimal, unlimited conditions, or in their relative efficiency of resource utilization under high density, limited growth conditions. Such differences result in a competitive advantage for one strain on the other, and may lead to an adaptive genetic shift in favour of the advantaged strain. The time required for genetic equilibrium depends on the degree of competitive advantage, as well as the magnitude of gene transfer and mutation rates. The relative importance of these factors in determining the timeframes of hypothetical adaptive shifts is discussed in Section 5.0.

1.3.5 Effects of Adaptive Changes on Tailings Geochemistry

Effects of local adaptive changes on tailings geochemical processes will depend on the speed with which these changes can become broadly established in the tailings. For example, a local change in an early successional species that might prevent the initial tailings acidification, would have little ultimate effect unless it was able to dominate the tailings prior to acidification. Once acidification sets in, early successional species would probably be replaced by acidophiles.

Thus, potentially important genetic events depend for their ultimate effect on their timing relative to the tailings acidification process, and the time required for adaptive shifts to spread throughout the tailings mass. These time constraints were considered for a number of hypothetical genetic events.

SECTION 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Literature Search Results

A large volume of literature was identified through computer literature database searches. Most of the pertinent material came from 1980 to present. Earlier studies were identified, both through the computer search and the references found in the more recent literature. However, it was found that research on bacterial oxidation of pyritic ores or tailings is a relatively new research topic. Thiobacillus ferrooxidans was first described by Colmer et al. (1950) as an iron oxidizing bacterium found in acid drainage from bituminous coal mines. Brierley (1982) indicated that Thiobacillus ferrooxidans was first associated with copper leaching in 1957. Brierley's review also indicated that, at the time (1982), little was known of either the genetics of leaching bacteria or of the mechanisms involved in leaching. We thus found the most relevant data for this study in the literature since 1980. It was also noted that, after the initial literature search, most subsequent searches and reviews of references listed in research publications duplicated titles already identified. This is a good indication that the majority of the relevant literature was identified in the search. The bibliography for this report (Section 7) presents the literature identified by the search.

2.2 Bacteria in Pyritic Tailings

A majority of the references found related to laboratory cultures. Such studies and the species or varieties researched may be of pertinence to development of optimal strains for leaching of ores, but they are only directly relevant to oxidation of pyritic ores if the species studied are naturally found in these ores. Where direct data are not available, laboratory results with related strains of bacteria can be used; however, it is important to assess the likelihood of the varieties reported in the literature being found in pyritic uranium mill tailings in order to determine the relevance of the data obtained. The following summarizes the bacterial species reported, along with discussions of their appropriateness to this study.

2.2.1 Thiobacillus ferrooxidans

This is the species most commonly associated with acid generation from pyritic ores (Lungren and Silver, 1980). It was first described in acidic coal wastewaters by Colmer

et al. (1950). It has also been found in pyritic coal wastes by Belly and Brock (1974), Martin et al. (1981) and Olson et al. (1981); in copper leaching solutions (Groudev et al., 1978; Groudev, 1980; Brierley, 1982); in copper/nickel ore leaching solutions (Trivedi and Tsuchiya, 1975); and in uranium ore leaching solutions (Guay et al., 1977; Tuovinen et al., 1981; DiSpirito et al., 1982).

Brock (1979) noted that most bacterial studies are done in the laboratory, since the complexity of mine tailings systems and the large variety of microorganisms present present major difficulties in designing and conducting a scientific field study. He further stated that this presents a significant problem since populations in soils (or tailings) exhibit a much lower activity level over longer time frames than those cultured in the laboratory under ideal conditions. Goodman et al. (1981a) felt that the nature of mine wastes makes a thorough sampling of the microenvironment difficult and costs prohibitive.

Goodman et al. (1981a, b) did conduct one of the more extensive studies of bacteria in uranium wastes in Australia. Thiobacillus ferrooxidans was the most consistently found species, and was most abundant in pyritic wastes and during the dry season. In wet seasons or non-pyritic wastes and overburdens, heterotrophic species dominated with 10 to 1,000 times greater populations. The bacteria that these authors identified in uranium wastes included most, if not all, of the recognized species of Thiobacillus as well as some novel species and Desulfovibrio spp. McCready (1976) and Silver and Taylor (1981) reported, in a similar manner, that, on the Nordic tailings at Elliot Lake, Ontario, T. ferrooxidans were rare in developed soils at vegetated sites and at moderate population levels in unvegetated areas. McCready found populations of 1.5×10^4 /g, while Silver and Taylor reported 1.9×10^3 /g, on the unvegetated tailings (pH 5.2 to 8). A variety of heterotrophs were present with populations of up to 4×10^4 /g on unvegetated or 7×10^7 /g on vegetated areas. Francis et al. (1980) reported bacteria on low level radioactive waste dumps. Harries and Ritchie (1983) also reported Thiobacillus spp. found on uranium mine waste rock piles. Medium populations of iron oxidizing bacteria (4.3×10^3 to 2.4×10^5) were reported in the upper 0.6 m of pyritic uranium mine tailings at the Lacnor site in Elliot Lake in field studies for the NUTP (Golder Associates, 1986).

2.2.2 Other Thiobacillus Species

The species most commonly associated with T. ferrooxidans in pyritic environments is T. thiooxidans. Belly and Brock (1974) found this species along with T. ferrooxidans in pyritic coal wastes. Brierley (1982) reported that mixed cultures of the two species leached metals best. She felt that the T. thiooxidans oxidizes S^0 to reduce Fe^{3+} and reduce pH, thus providing a more optimal medium for T. ferrooxidans to grow in. Brock and Gustafson (1976) and Hoffman *et al.* (1981) reported similar conclusions. Mizoguchi and Okabe (1980) reported T. thiooxidans to be a mixotrophic or heterotrophic species which, in mine water, requires a carbon energy source. This species is not directly involved in oxidation of ore. Goodman *et al.* (1981a, b) found no T. thiooxidans in a variety of waste rock, soil and pond studies at an Australian uranium mine site.

T. rubellus and T. delicatus have been reported in mine runoff waters (Mizoguchi and Okabe, 1980). T. perometabolis has been reported from a pyritic coal mine (Myers and Miller, 1975). T. acidophilus has also been found in acidic mine waters (Arkesteyn and DeBont, 1980). None of these species have been given the importance in acid generation from pyritic tailings that has been reported for T. ferrooxidans. Another species, Thiobacillus albertis, has been reported from the vicinity of a sulphur pile. Goodman *et al.* (1981a, b) noted that almost all known Thiobacillus species were found in wastes at a uranium mine site in Australia.

2.2.3 Other Bacterial Genera

The genus Metallogenium is speculated to have some importance in the initial stages of pyritic ore oxidation (Walsh and Mitchell, 1972a, b; Lundgren and Silver, 1980; Harrison, 1978). It functions at pH levels greater than 4.5, reducing the pH in the tailings to levels at which T. ferrooxidans can successfully replace it. Trivedi and Tsuchiya (1975) reported a mutualistic relationship in copper-nickel leaching solutions between T. ferrooxidans and the nitrogen fixing bacterium Beijerinckia lacticogenes. Lundgren and Silver (1980) felt Gallionella spp. might also be important in succession.

Acidiphilium cryptum has been reported in coal (Harrison, 1981; Wichlacz and Unz, 1981) and copper piles (Harrison, 1981). Goodman *et al.* (1981a) reported Desulfovibrio spp. in waste piles at a uranium mine site. McCready (1976) and Silver and Taylor (1981) reported high populations of heterotrophs in vegetated tailings at Elliot Lake, Ontario.

Other species have been reported from hot springs, such as Sulfolobus acidocaldarius (Bohlool and Brock, 1974; Brierley, 1982) and Thermothrix spp. (Brierley, 1982); or from burning rubbish (Thermoplasma acidophilum) (Stein and Searcy, 1978), but not from mine tailings.

Lundgren and Silver (1980) noted that, besides the dominant T. ferrooxidans, there were a variety of minor heterotrophic bacteria, fungi, yeasts, algae and protozoans in pyritic tailings. Groudev et al. (1978) found a number of genera in copper leach solutions, including Thiobacillus, Gallionella, Leptothrix, Desulfovibrio, Bacillus, Enterobacter, Pseudomonas and Caulobacter, as well as molds, yeasts, algae and protozoa. Groudev (1980) and DiSpirito et al. (1982) actually felt that T. ferrooxidans was a number of related species rather than just one.

2.3 Potential Gaps in Existing Data

It must be emphasized that any attempt to model the influence of genetic and adaptive changes on the growth of bacterial populations and the effect of this on acidification of uranium mill tailings will be based on a wide variety of speculative assumptions at this time. We have attempted to present the best approach based on an extensive review of existing data. However, many assumptions have had to be made based on species of bacteria not associated with tailings, or even on higher organisms. In order to emphasize the speculative nature of some of the assumptions made in our model as well as to point out areas of needed research, some of the major data gaps or interpretation problems are presented below.

2.3.1 Uranium Mill Tailings Studies

Few studies were found of bacterial species in uranium mill tailings. The National Uranium Tailings Program studies of Gunnar and Lacnor sites (BEAK, 1985b; Golder Associates, 1986) did look at populations of aerobic, anaerobic, sulphate reducing, sulphur oxidizing and iron oxidizing bacteria. These were not identified to species. Three studies of the Agnew Lake, Ontario uranium mine leach liquors (Guay et al., 1977; Tuovinen et al., 1981; DiSpirito et al., 1982) identified Thiobacillus ferrooxidans as the main acidification species. Several of the co-authors are the same for these three studies. Studies of bacterial populations in uranium mill waste areas were reported by

McCready (1976), Francis et al. (1980), Goodman et al. (1981a, b) and Silver and Taylor (1981). The McCready and Silver and Taylor studies were of old, partially rehabilitated tailings with relatively high pH (5 to 8) levels, and were dominated by heterotrophs.

The majority of studies on Thiobacillus and other genera relevant to pyritic uranium mill tailings acidification are with cultures of bacteria which have been maintained in the laboratory for many generations on special media. The bacteria have adapted to the media which, in most cases, means they have evolved genetically from the original wild strains (Strauss et al., 1985). Most of the data on growth rates, mutation rates, nutritional requirements, toxic sensitivities, etc. are derived from carefully controlled, laboratory experiments where media and the stresses imposed are not directly comparable to uranium mill tailings. These laboratory cultures have also resulted in a wide variety of Thiobacillus (and other genera) species which have never been isolated from field samples (c.f. Matin, 1978; Stein and Searcy, 1978; Martin et al., 1981; Brierley, 1982). Brock (1979) emphasized that laboratory culture studies on ideal media provide conditions for much higher activity levels than in natural soils and tailings.

2.3.2 Bacterial Genetics and Phylogeny

Because of the size of the organisms, their life cycle, multitude of genetic transfer mechanisms, rapid mutation and short generation time, bacterial genetics are poorly understood. Little is known of the genetics of bacteria involved in the leaching of pyritic ores, nor of the exact mechanisms they use to degrade these minerals (Summers and Silver, 1978; Brierley, 1982). Both Groudev (1980) and DeSpirito et al. (1982) have suggested that Thiobacillus ferrooxidans is not a species, but is actually a community with a number of physiologically or metabolically similar species working together. Strauss et al. (1985), in their recent review, agree that microorganisms in nature function as a community not as individuals. They note further that the high diversity and high rate of mutagenesis in bacterial communities mean that a natural population will have a high number of new strains appearing, some of which will successfully integrate into the community, others of which may persist for long periods at very low densities.

This means that the strains of Thiobacillus ferrooxidans found in any one uranium mill tailings may be physiologically different from those found on similar tailings elsewhere (c.f. Martin et al., 1981). Since bacteria are studied by growing populations on culture

media until they become large enough to experiment with, much of the natural genetic variation may be lost in the laboratory due to the competitive edge given one strain or variety by the contents of the media (c.f., Mizoguchi and Okabe, 1980).

Strauss et al. (1985) provide an extensive review of the state-of-the-art of knowledge on bacterial genetics and phylogeny. The following quotes emphasize their feelings:

- o "The existing system of bacterial classification is such a poor reflection of natural (phylogenetic) relationships among bacteria that it virtually excludes evolutionary considerations.....".
- o "Bacteria are not subject to genetic isolation and thus cannot be considered to be divided into true species..... Their genetic exchange processes are completely separate from their processes of reproduction". In other words, plasmid exchange, transformation, viral transduction, chromosomal conjugation and transposon activity break down the classical definition of a species which is based on genetic barriers.
- o "Systems of microbial taxonomy are currently in disarray."

Strauss et al. (1985) summarize the major unknowns relative to bacterial genetics and the effects of mutations on wild populations as:

- o how DNA is altered or exchanged between organisms and what the effects are;
- o the survival and growth rates of new organisms;
- o transportation and dispersion rates for new organisms in the environment; and
- o if a new organism survives, will it transfer genetic information.

2.3.3 Laboratory Mutagenic Studies

Most information on mutagenesis in Thiobacillus and other bacteria comes from laboratory studies involving known mutagenic agents or other unnatural stresses (c.f., Bruynesteyn et al., 1980a, b; Soljanto and Tuovinen, 1980; Olson et al., 1982). Mutation rates in the field are assumed to be similar to those measured in the laboratory without the use of mutagenic agents.

2.3.4 Plasmids

Plasmids appear to be the most effective mechanism for genetic transfer between bacteria (Barros et al., 1985; Rawlings and Woods, 1985; Strauss et al., 1985). They have also been associated with important traits in Thiobacillus ferrooxidans such as uranium toxicity resistance (Martin et al., 1981; DiSpirito et al., 1982), ferrous iron oxidation (Summers and Silver, 1978; but Martin et al., 1981 disagree), and resistance to other metal toxicities (Davidson and Summers, 1983; Davidson et al., 1985; Hurtado and Bauer, 1985).

Many of the plasmids studied in Thiobacillus originate from Escherichia coli (Martin et al., 1981; Davidson et al., 1985; Davidson and Summers, 1985), and thus these specific plasmids may not be found in wild strains of T. ferrooxidans. However, Martin et al. (1981) found that 11 of 15 strains of T. ferrooxidans carried native plasmid DNA. Each of these strains had a different plasmid composition. Very little information exists on native plasmid genetics or transfer under field conditions.

Some confusion in plasmid identification is emphasized by Martin et al. (1984) who found that the plasmid they were studying in Sulfolobus was actually a virus. Yeats et al. (1982) also felt that plasmids and viruses were often confused.

2.4 Potential Mutagenesis Caused by Ionizing Radiation

The following discussion is largely taken from an in-house review of bacterial mutation in uranium tailings by the National Uranium Tailings Program (Weatherell, 1985).

Following Muller's pioneering work with x-rays and Drosophila (fruit flies) in 1927, the next two decades of genetic research were dominated by research on mutagenic properties of ionizing radiation (Auerbach, 1976). Continued research indicated that:

- o exposure to high levels of ionizing radiation increases mutation rates substantially (15,000%), but survival rates were low (less than 0.1%);
- o induced mutation rate increases linearly with dose; and
- o survival rate and the number of viable mutants decrease (linearly and exponentially, respectively) with dose near lethal dose levels.

Although there is a lack of studies of bacteria in uranium mill tailings, research to date indicates the levels of radioactivity typical for uranium mill tailings are insufficient to cause any significant increase in mutation rates (Silver and Taylor, 1981). Typical levels of radioactivity in pore water from Elliot Lake tailings range from 37 to 370 mBq/mL (Halbert et al., 1984). Experiments with bacteria (Francis et al., 1980) have indicated that exposure to 100 Bq/mL of ionizing radiation resulted in no significant increase in the mutation rate, and that exposure levels of up to 100,000 Bq/mL were necessary to cause appreciable increases in mutation.

Radiation mutagenesis is considered to be a stochastic effect. Thus, there is likely to be a slight increase in mutation rate as a result of radioactivity in the tailings. From radionuclide levels in Lacnor porewater (Golder Associates, 1986), a radiation dose rate of approximately 2.3×10^{-4} uGy/h would be reasonable for microorganisms in tailings, based on the IAEA (1976) phytoplankton model, and assuming porewater incorporation by the organisms. Heddle and Athanasiou (1975) report a bacterial-induced mutation rate of about 10^{-7} mutations/locus/Gy, or about 5.75×10^{-16} induced mutations/locus/generation (25 hr) as a result of tailings radiation. This increment is not biologically significant relative to spontaneous mutation rates on the order of 10^{-8} .

SECTION 3

GENERAL METABOLISM AND ECOLOGY

3.0 GENERAL METABOLISM AND ECOLOGY OF ACIDOPHILIC BACTERIA

In order to determine the potential effect of new strains of bacteria on the rate of generation of acid through pyrite oxidation, one has to know the metabolic and growth characteristics and requirements of the species involved and how these relate to the tailings environment. Pertinent data from the literature are discussed below.

3.1 Oxygen Requirements

Belly and Brock (1974) indicated that oxygen levels were adequate to support populations of T. ferrooxidans and T. thiooxidans in the top 10 cm of tailings. It has also been a long-established theory that underwater disposal of tailings which prevents direct access of the pyrite oxidizing bacteria to oxygen will reduce or prevent acid generation (Hawley, 1975). Goodman et al. (1981a) reported that T. ferrooxidans populations decreased significantly during wet seasons on uranium mine wastes in Australia. Silver and Torma (1974) found that T. ferrooxidans grown on FeSO_4 and then tested on pyritic substrate utilized 30 $\mu\text{mol/h}$ of oxygen.

Some results were also found for other species. Myers and Miller (1975) reported oxygen uptake levels for T. perometabolis of 23 $\mu\text{L/mL medium/h}$. Justin and Kelly (1978a, b) reported T. denitrificans to grow anaerobically. Walsh and Mitchell (1983) found maximum oxygen uptake rates for Metallogenium spp. after two hours of culturing in 40 mg/L ferrous iron medium to be 2 $\mu\text{L/mL medium/h}$. Higher or lower iron concentrations inhibited oxygen uptake.

Guay et al. (1977) reported maximum ferrous iron oxidation rates for T. ferrooxidans of 15.0 mmol/L/h during the late logarithmic growth phase. Silver and Torma (1974) reported total oxygen uptake for T. ferrooxidans on pyrite medium of slightly over 300 $\mu\text{L/mL medium/h}$.

None of the above actually provide a limiting level of O_2 for T. ferrooxidans survival or growth, nor do they relate rates of population increase to levels of O_2 available. Equations for oxygen limitation in an open system are suggested by SENES (1986). Oxygen in the tailings environment ranges from about 20% at the tailings surface to 0% at the anaerobic horizon, declining exponentially with depth.

3.2 pH Optimum and Tolerance

Literature values for pH optima and tolerance ranges for Thiobacillus spp. and other genera are presented in Table 3.1. McCready (1976) and Silver and Taylor (1981) found that, in surface areas of old pyritic tailings where revegetation was occurring and pH levels were close to neutral (5 to 8), T. ferrooxidans populations were low and overshadowed by heterotrophic species. pH in an acidified tailings mass may range from about 1 at the tailings surface to 6.5 at the bottom of the aerobic zone where acidifying bacteria are inactive.

3.3 Temperature Optimum and Tolerance Range

Temperature optimal ranges found in the literature for Thiobacillus spp. are presented in Table 3.2. Soljanto and Tuovinen (1980) noted that T. ferrooxidans generate a lot of heat during oxidation of Fe^{+2} to Fe^{+3} ($\Delta G^1 = -74.4 \text{ kJ/mol}$) and U^{+4} to U^{+6} ($\Delta G^1 = -86.2 \text{ KJ/mol}$). This could create a local microclimate effect where particle surfaces in the upper layers of active tailings are much hotter than areas lacking bacterial activity.

Tailings temperature varies seasonally and with depth, seasonal variations being most pronounced in the upper layers. A typical seasonal temperature range in the upper layers would be from less than 0°C to about 30°C , excluding particle surface effects. Psychrotrophic strains of T. ferrooxidans capable of activity at 0°C have been isolated (Ferroni et al., 1986).

3.4 Growth Rates and Carrying Capacities

Growth rates and carrying capacities from the literature are documented in Tables 3.3 and 3.4. Belly and Brock (1974) noted that, for a new tailings environment in pyritic coal wastes, two to three years of time was required before significant populations (determined by CO_2 generation) of T. ferrooxidans and T. thiooxidans were built up. This lag phase may represent the time required for chemical or successional acidification to produce conditions suitable for growth of thiobacilli.

Growth rates vary as a function of pH and temperature, and are highest in the optimum range. Substrate composition may also be important. Mixotrophic environments may enhance the growth of some thiobacilli, such as T. perometabolis (Myers and Miller,

TABLE 3.1: OPTIMAL AND TOLERATED pH LEVELS

Bacterial Species	Optimal pH	pH Tolerance Range	Reference
<u>T. ferrooxidans</u>	-	1.7 to 4.6	Silver and Torma, 1974
<u>T. ferrooxidans</u>	2.3 to 2.8	-	Groudev, 1980
<u>T. ferrooxidans</u>	2.3 to 2.7	0.8 to -	Bruynesteyn <u>et al.</u> , 1980a, b
<u>T. ferrooxidans</u>	2.5 to 2.8	-	Bryant <u>et al.</u> , 1983
<u>T. thiooxidans</u>	2.0 to 3.5	-	Bryant <u>et al.</u> , 1983
<u>T. kabobis</u>	3.0	-	Bryant <u>et al.</u> , 1983
<u>T. acidophilis</u>	3.0	-	Bryant <u>et al.</u> , 1983
<u>T. albertis</u>	3.5 to 4.0	-	Bryant <u>et al.</u> , 1983
<u>T. ferrooxidans</u>	1.4	-	Belly and Brock, 1974
<u>T. thiooxidans</u>	1.4	-	Belly and Brock, 1974
<u>T. ferrooxidans</u>	L 2*	-	Norris and Kelly, 1978
<u>Leptospirillum</u> spp.	L 2	-	Norris and Kelly, 1978
<u>Thiobacillus</u> spp.	L 4.5	-	Lundgren and Silver, 1980
<u>Gallionella</u> spp.	G 4.5*	-	Lundgren and Silver, 1980
<u>Metallogenium</u> spp.	4.5	-	Lundgren and Silver, 1980
<u>T. intermedius</u>	4 to 8	2.5 to -	Harrison, 1983
<u>T. rapidocrasans</u>	7.5 to 9	6.5 to 9.5	Katayama-Fujimura <u>et al.</u> , 1983
<u>T. novellus</u>	7 to 7.4	5.7 to 9	Katayama-Fujimura <u>et al.</u> , 1983
<u>T. perometabolis</u>	5.5 to 6	5 to 7.5	Katayama-Fujimura <u>et al.</u> , 1983
<u>T. perometabolis</u>	5.5 to 6	5 to 7.5	Katayama-Fujimura and Kuraishi, 1983
<u>T. intermedius</u>	5.5 to 6	5 to 7	Katayama-Fujimura <u>et al.</u> , 1984
<u>T. delicatus</u>	5.5 to 6	5 to 7	Katayama-Fujimura <u>et al.</u> , 1984
<u>T. perometabolis</u>	6	3 to -	Myers and Miller, 1975

* L = less than

G = greater than

TABLE 3.2: TEMPERATURE OPTIMA REPORTED FOR GROWTH OF
THIOBACILLUS sp.

Species	Optimal Temperatures (°C)	Reference
<u>T. ferrooxidans</u>	28	Belly and Brock, 1974
<u>T. thiooxidans</u>	28	Belly and Brock, 1974
<u>T. ferrooxidans</u>	15 to 20	Bryant <u>et al.</u> , 1983
<u>T. thiooxidans</u>	28 to 30	Bryant <u>et al.</u> , 1983
<u>T. kabobis</u>	28	Bryant <u>et al.</u> , 1983
<u>T. acidophilus</u>	25 to 30	Bryant <u>et al.</u> , 1983
<u>T. albertis</u>	28 to 30	Bryant <u>et al.</u> , 1983
<u>T. ferrooxidans</u>	35 to 37	Groudev, 1980
<u>T. delicatus</u>	30	Mizoguchi and Okabe, 1980
<u>T. rapidocrescens</u>	30 to 37	Katayama-Fujimura <u>et al.</u> , 1983
<u>T. novellus</u>	25 to 30	Katayama-Fujimura <u>et al.</u> , 1983
<u>T. perometabolis</u>	35 to 37	Katayama-Fujimura <u>et al.</u> , 1983
<u>T. intermedius</u>	30 to 35	Katayama-Fujimura <u>et al.</u> , 1984
<u>T. delicatus</u>	30 to 35	Katayama-Fujimura <u>et al.</u> , 1984
<u>T. novellus</u>	25 to 30	Katayama-Fujimura <u>et al.</u> , 1980

TABLE 3.3: POPULATION CARRYING CAPACITIES FROM THE LITERATURE

Species	Carrying Capacity (cells)	Reference
<u>T. ferrooxidans</u>	$G * 10^6/g$	Brierley, 1982
<u>T. ferrooxidans</u>	$6 \times 10^7 - 6 \times 10^9/g$	SENES, 1986
<u>T. thiooxidans</u>	$L * 10^6/g$	Brierley, 1982
<u>T. albertis</u>	$4 \times 10^8/mL$	Bryant <u>et al.</u> , 1983
<u>T. ferrooxidans</u>	$3 \times 10^8 - 22 \times 10^8/mL$	Sugio <u>et al.</u> , 1981a
Thiobacilli	$10^8/g$	Belly and Brock, 1974
Iron-oxidizers	$10^6/g$	Golder Associates, 1986

* G = greater than.

L = less than.

1975), with broad substrate ranges. A mixotrophic strain of T. ferrooxidans has been reported (Barros et al., 1984), although there is no indication from the reported laboratory study that mixotrophy confers any selective advantage to the isolate in its natural environment.

3.5 Generation Times

Generation times reported in the literature, or calculated from exponential phase growth kinetics, are presented in Table 3.4.

3.6 Substrate Utilization and Tolerance Levels

T. ferrooxidans has been reported to grow on pyritic coal wastes (Colmer et al., 1950; Belly and Brock, 1974; Olson et al., 1981), copper leach solutions (Brierley, 1982; Groudev et al., 1978), and uranium leaching solutions (Guay et al., 1977; Ralph and Khalid, 1981a; Silver, 1981; Tuovinen et al., 1981; DiSpirito et al., 1982), as well as being the main bacterial species associated with acid generation in pyritic tailings (Lungren and Silver, 1980; Halbert et al., 1984). The genus Thiobacillus is autotrophic, or able to grow with minimal nutrients, an ability which is essential for life in a low organic carbon tailings environment (Strauss, 1985). Although usually associated with iron oxidation, T. ferrooxidans can reduce Fe^{+3} to Fe^{+2} using S^0 as an energy source (Brock and Gustafson, 1976). This latter reaction is so slow though that it is masked by the former.

Martin (1978) reports that T. ferrooxidans is strongly inhibited by organic and amino acids at concentrations as low as 0.1 mmol/L. Thus, its activity could easily be curtailed by organic waste accumulation. However, Billheimer and Blanchard (1981) and Sugio et al. (1981) report some strains with an ability to utilize organic substrates facultatively.

Groudev (1980) reported substrate inhibitory levels for metal ions in tailings to two strains of T. ferrooxidans as copper 25 to 30, zinc 10 to 12, nickel 10, cobalt 5 to 8 and molybdenum 0.04 to 0.05. The effect is somewhat confusing since the units of the inhibitory concentrations are expressed as g^{-1} . Guay et al. (1977) discuss a number of factors which increase iron oxidation rates by T. ferrooxidans in low grade uranium ores, including aeration, agitation and ferrous ion concentration. Oxidation was inversely proportional to ore particle size. Hoffman et al. (1981) reported similar substrate

TABLE 3.4: GENERATION TIMES AND GROWTH RATES FROM THE LITERATURE

Species	Time (hours) per Generation	Growth Rate	Reference
<u>T. ferrooxidans</u>	4.5 to 6.4	0.108 to 0.155	Groudev, 1980
<u>T. ferrooxidans</u>	6.5 to 17	0.041 to 0.107	McGoran <u>et al.</u> , 1969
<u>T. intermedius</u>	25	0.028	Harrison, 1983
<u>T. perometabolis</u>	16.7	0.042	Harrison, 1983
<u>T. thioparus</u>	3.3	0.210	Stark and Yanofsky, 1981
<u>T. novellus</u>	8.0 to 11.2	0.062 to 0.087	Perez and Matin, 1980
<u>T. albertis</u>	13.1	0.053	Bryant <u>et al.</u> , 1983

effects on both T. ferrooxidans and T. thiooxidans. Optimal growth occurred with a nitrogen/phosphorus ratio of 90:1 and a substrate particle size less than 43 μm . Chromium, cadmium and boron inhibited oxidation rates. Tuovinen and Kelly (1974) adapted T. ferrooxidans to increasing concentrations of uranium up to 5 mM UO_2 by subculture methods.

T. thiooxidans is associated with T. ferrooxidans, but at slightly smaller population levels in pyritic ores or tailings (Belly and Brock, 1974). It appears to have similar tolerance levels to toxic metal ions (Hoffman et al., 1981), but a greater ability to use elemental sulphur or sulphur compounds as substrates.

Mizoguchi and Okabe (1980) noted that T. thiooxidans were adversely affected by Hg^{+2} at over 10 mg/g and Ag^+ at over 0.5 mg/g without Fe^{+3} in copper ore. They also reported that, with Fe^{+3} present, 0.5 mg/g Ag^+ increased oxidation rates, and rates were not affected significantly by the following concentrations: Ag^+ (0.1 mg/g), Pb^{+2} (10 mg/g), Cu^{+2} (10 mg/g), Cd^{+2} (10 mg/g) and Ni^{+2} (10 mg/g). Goodman et al. (1981b) reported T. thiooxidans to have a lower tolerance of many metal ions than T. ferrooxidans.

Less information was found on other bacterial species. T. albertis can grow on substrates including S^0 , S_2O_3 or S_4O_6 (Bryant et al., 1983). T. delicatus has been found in mine waters, but could not be maintained for any length of time on cultures (Mizoguchi and Okabe, 1980). Metallogenium spp. has been found in coal and zinc ores (Walsh and Mitchell, 1972) and requires the presence of ferrous iron. A. cryptum is a heterotrophic species requiring a glucose (or carbon) source (Harrison, 1983), but it has been found in coal and copper leaching solutions (Norris and Kelly, 1978). T. perometabolis has been found in coal mine wastes (Myers and Miller, 1975).

3.7 Geochemical Role

T. ferrooxidans and T. thiobacillus both seem to play key roles in the oxidation of pyritic Fe^{+2} to Fe^{+3} since the biotic reaction is 10^6 times faster than the abiotic. Thus, the bacteria can accelerate leaching, acidification and metal mobilization processes (Belly and Brock, 1974; Brierley, 1982; Brock and Gustafson, 1976).

Other species apparently play roles in initiating the lowering of tailings pH levels to those optimal for T. ferrooxidans. These include Metallogenium spp. (Belly and Brock, 1974), T. intermedius and A. cryptum (Harrison, 1983). Nitrogen fixation by Beijerinckia lacticogenes and reduction by T. delicatus have also been reported in pyritic ores (Trivedi and Tsuchiya, 1975; Katayama-Fujimura et al., 1984).

3.8 Species Interactions and Succession

Mixed cultures of T. ferrooxidans and T. thiooxidans have been reported to oxidize pyritic materials faster than pure cultures of either species (Norris and Kelly, 1978; Hoffman et al., 1981; Wakao et al., 1982; Brierley, 1982). Brierley explains that T. thiooxidans use S^0 and some sulphur compounds in a neutral environment resulting in a more rapid reduction in pH to levels at which T. ferrooxidans functions best. Brierley (1982) also refers to mixed cultures of Leptospirillum ferrooxidans and T. organoparus having the ability to oxidize pyrites or chalcopyrites while pure cultures of either species cannot. Norris and Kelly (1978) note a similar mutualistic interaction between L. ferrooxidans and T. ferrooxidans, with the activity of each species enhanced in mixed culture. Harrison (1981) felt that A. cryptum used organic matter produced by T. ferrooxidans, thereby removing inhibitory waste material and enhancing T. ferrooxidans growth. Similarly, Beijerinckia lacticogenes, a nitrogen-fixing heterotroph, seems to increase the oxidation rate of Cu-Ni sulphide by T. ferrooxidans (Trivedi and Tsuchiya, 1975). DiSpirito et al. (1982) feel that what many authors refer to as T. ferrooxidans is actually a highly integrated community of related and ecologically associated species.

Harrison (1983) notes that T. intermedius and A. cryptum both have successional roles in reducing pH to levels that more acidophilic species need to function. Lundgren and Silver (1980) and Belly and Brock (1974) refer to similar roles for Gallionella spp. and Metallogenium spp. However, Arkesteyn (1980) suggests that this initial acidification may be primarily non-biological.

3.9 Uranium Mill Tailings Environment

In order to match the bacterial preference or tolerance parameters discussed above with the actual environment found in uranium mill tailings, data on the average environment

found in pyritic tailings from the Elliot Lake area are presented in Table 3.5. Pyrite concentrations in ore particles may vary from about 1 to 7%.

The most important features of the tailings environment, from the point of view of limiting effects on bacteria, are strongly depth-dependent. For example, oxygen concentration in the tailings decreases exponentially with depth as a function of porosity and moisture saturation. Oxygen may range from about 20% at the tailings surface to 0% in the anaerobic saturated zone. Bacterial activity also influences oxygen concentration.

pH varies with depth, primarily as a function of oxidation rate and pore water movement once acidification has been initiated. Near the tailings surface, a pH near 1 may occur, increasing to about 7 below the zone of active oxidation and the zone where solid buffer material is depleted.

Temperature varies cyclically at depth with the seasons. In the summer months, temperatures are highest near the surface (ca. 30°C), and during the winter months they are lowest near the surface (below 0°C). The seasonal temperature fluctuation decreases with depth.

Temperature, pH and oxygen effects dictate that most bacterial activity and, therefore, the greatest potential rates of adaptive change occur near the tailings surface, given that there is sufficient surface moisture. Moisture tends to increase with depth, and is a key factor limiting oxygen penetration.

TABLE 3.5: CONCENTRATIONS OF ELEMENTS IN PORE WATER OF ELLIOT LAKE TAILINGS (Beak Consultants Limited and Golder Associates, 1986)

	Quirke Tailings Discharge*	Sandy		Silty	
		Tailings Depth		Tailings Depth	
		1 m**	9 m***	1 m**	9 m***
pH	10.9	1.4	5.8	2.4	5.9
Fe (total)	1.4	23,038	12,520	3,343	3,275
Al (ppm)	3.0	230	0.8	41.2	1.6
SiO ₂ (ppm)	-	33.0	40.4	168	27
Ca (ppm)	612	734	400	484	396
Mg (ppm)	0.35	15.6	460	19	246
Na (ppm)	36	11.9	14	3.3	3.7
K (ppm)	142	123	170	30	135
SO ₄ (ppm)	1,325	162,000	28,000	2,870	7,810
PO ₄ (ppm)	-	L 2.0	-	L 2.0	-
Cl (ppm)	14	24	4.9	6.4	1.0
F (ppm)	-	-	L 2.0	L 2.0	3.4
Cu (ppm)	0.06	11.3	0.05	4.4	L 0.01
Zn (ppm)	0.04	15.9	7.3	2.1	1.0
Ni (ppm)	0.09	27.1	16	3.6	1.3
Co (ppm)	0.09	34.9	14	3.9	1.9
Pb (ppm)	0.17	-	0.67	-	0.51
Mn (ppm)	0.04	1.5	15	0.7	95
Alk (as HCO ₃) (ppm)	-	-	232	-	165
Ra Total (pCi/L)	1,679	-	132	-	0.60
NO ₃ (ppm)	575	-	-	-	-

* Analysis done by Rio Algom Laboratory, March 1977, A.J. Vivyurka, written communication (data from Dubrovsky *et al.*, 1985a).

** Data from Smyth (1981).

*** Data from Cherry *et al.* (1982).

SECTION 4

STRAIN VARIATION AND POTENTIAL ADAPTIVE CHANGES

4.0 STRAIN VARIATION AND POTENTIAL ADAPTIVE CHANGES

Most microbial species probably include a diverse collection of strains which differ from one another in their metabolic characteristics, as a result of their genetic differences. Strains which belong to the same species tend to be more similar to each other genetically than strains belonging to different species, however microbial species have not been defined in a manner that necessarily reflects genetic relatedness (Stackebrandt and Woese, 1984). Genetic exchanges between strains of the same and different species adds to the complexity of genetic relationships. New strains may arise either by mutation, or by recombination of genetic elements from previously existing strains. Thus gene transfer between strains may be an important factor in microbial evolution, as well as a complicating factor in microbial taxonomy.

Constraints on gene transfer do exist, although extra-chromosomal genetic elements (e.g., plasmids) are easily transferred between unrelated species. Roberts (1976) and Datta (1979) review the barriers to gene transfer, including plasmid incompatibility systems. These molecular mechanisms ensure that certain combinations of genetic elements do not occur, or occur rarely, thus permitting recognition of the combinations that do occur as reasonably discrete entities, or strains.

Slater and Godwin (1980) review the mechanisms of microbial adaptation and selection, including the role of plasmid DNA. Campbell (1981) focuses specifically on the evolutionary role of extra-chromosomal DNA. Both authors point out that plasmid transfer can be an important mechanism in the spread of new strains, as well as in the creation of new strains. Reanny (1976, 1977, 1978) suggests that extra-chromosomal elements may be the primary mechanism of genetic plasticity through which microbial adaptive processes work. They are certainly important in laboratory environments, and are transferred in natural environments (Weinberg and Stotzky, 1972; Graham and Istoch, 1979), however, there is little quantitative data addressing their importance in nature.

Campbell (1981) argues that natural selection should favour the occurrence of genes which are needed only occasionally on plasmids, while genes needed regularly should be incorporated into the chromosomal DNA. Since chromosomal DNA is less mobile, it tends to become more highly integrated, and thus better protected by incompatibility systems. This would account for the greater phylogenetic limitations on interspecific chromosomal transfer.

Resistance factors, which are needed only on occasions when environmental toxicants reach extreme levels, do tend to be carried on plasmids. Many microbial strains are identified on the basis of resistance characteristics, and are therefore plasmid-mediated strains. Strains resistant to various metal ions are particularly common in Thiobacillus (Table 4.1). However, strains identified on the basis of chromosomal characteristics are likely to be more stable.

Plasmids replicate rapidly on entry into a new host organism and hence are usually carried in multiple copies. This replication utilizes energy resources, and represents a metabolic burden to the host cell. Thus, natural selection should eventually eliminate plasmids which do not occasionally confer some advantage to the host cell. However, plasmids with a high rate of transfer can be maintained in the population for long periods in the absence of any apparent selective value. This persistence may be of value to the population as a whole, since it greatly expands the gene pool from which new characteristics may be acquired when environmental changes make new characteristics useful. Campbell (1981) even suggests the possibility that certain strains may be able to act as genetic reservoirs, harbouring plasmids of low selective value.

4.1 Growth and Oxidation Rates

In mixed communities, microbial strains with the greatest finite (i.e., actual) growth rates enjoy competitive superiority (Slater and Godwin, 1980). However, high finite growth rates may arise in several ways. Activity of the rate limiting enzyme may be high, resulting in a high intrinsic growth rate, or the affinity of the rate-limiting enzyme for its substrate may be high, resulting in greater energetic efficiency. Efficiency is important mainly at low substrate concentrations.

Kuenen et al., (1977) and Matin and Valkamp (1978) both describe competitive systems where one strain is superior under ample substrate (high growth) conditions, while another strain becomes superior under limited substrate (low growth) conditions. The first strain has the higher intrinsic growth rate, while the second strain is more efficient. Quantitative estimates of intrinsic growth and efficiency under ambient environmental conditions are required for prediction of adaptive responses and their time frames.

TABLE 4.1: GROWTH/OXIDATION CHARACTERISTICS OF MUTANT THIOBACILLI

Species (Strain)	Growth/Oxidation Characteristics	Reference
<u>T. thioparus</u> (SNT-F)	generations/hr 5-10 times parental strain	Stark and Yanofsky, 1981
<u>T. thioparus</u> (SNP-F) Natural mutants	at low substrate concentration equal to parent at high substrate	
<u>T. novellus</u> (MD134)	S ⁰ oxidation 68 to 70% of parent	Davidson <u>et al.</u> , 1985
<u>T. novellus</u> (MD146) Transposon mutants	S ₂ O ₃ ⁻² oxidation 40 to 48% of parent CO ₂ fixation 50% of parental strain	
<u>T. ferrooxidans</u> (??) UV mutant	S oxidation improved over parental strain	Groudeva <u>et al.</u> , 1981 (in Torma, 1983)
<u>T. ferrooxidans</u> (??) Natural mutant	O ₂ uptake improved over parental strain, associated with U resistance	Dispirito and Tuovinen, 1981
<u>T. ferrooxidans</u> (SU-11) Natural mutant	intrinsic growth rate 130% of wild strain	Gorog <u>et al.</u> , 1980 (in Weatherell 1985)
<u>T. ferrooxidans</u> (pos)	Fe oxidation 160% of parental strain	Groudev, 1978 (in Weatherell 1985)
<u>T. ferrooxidans</u> (neg)	Fe oxidation 80% of parental strain	
<u>T. ferrooxidans</u> (M-8-1)	Fe oxidation 143% of parent M-8	Groudev, 1980

Weatherell (1985) presents kinetic constants from the laboratory growth data of Gorog *et al.*, (1980) for four strains of Thiobacillus ferrooxidans isolated from copper mill tailings in Hungary. Intrinsic growth rates ranged from 0.11 to 0.15 hr⁻¹ and maximum cell numbers at substrate saturation, from 2.85x10⁸ to 3.50x10⁸ cm⁻³. These data suggest a correlation between efficiency, as represented by maximum cell numbers (i.e., bacterial carrying capacity), and intrinsic growth rate.

A possible explanation is provided by Hartley (1974) who suggests that gene doubling may be a necessary pre-requisite for subsequent evolution of structurally modified enzymes. Gene doubling is often the cause of increased enzyme activity and intrinsic growth (Slater and Godwin, 1980), while structural changes are required for increased enzyme-substrate affinity and efficiency.

Population growth kinetics are closely associated with the chemical kinetics of substrate oxidation. Weatherell (1985) illustrates the correlation between intrinsic growth rate and iron oxidation rate using the data of Gorog *et al.*, (1980) for T. ferrooxidans strains. Maximum oxidation rates range from 190 mg Fe²⁺/L/hr (strain F-4, Groudev, 1978) to 347 mg Fe²⁺/L/hr (strain M-8-1, Groudev, 1980). Some growth and oxidation characteristics of mutant Thiobacillus strains are compared to parental or wild strains in Table 4.1

Stark and Yanovsky (1981) describe an enhanced growth mutant of T. thioparus that differs from the parental strain only at low (limiting) substrate concentrations. This represents an example of enhanced efficiency of substrate utilization.

DiSpirito and Tuovinen (1981) describe a uranium-resistant T. ferrooxidans mutant with enhanced growth as a result of increased oxygen uptake. Oxygen uptake has a general effect on oxidation rates unrelated to any changes in oxidizing enzymes. Campbell (1981) points out that oxidation capabilities may be related to detoxification reactions, rather than energy metabolism. Thus, uranium resistance in this strain may be related to enhanced growth characteristics.

4.2 Substrate Utilization

The range of substrates utilized by microbial strains can influence the degree of competition with other strains. Some examples of Thiobacillus strains with different

substrate capabilities than the wild type are listed in Table 4.2. Utilization of a substrate not used to the same degree by other strains, can prevent replacement of an otherwise inferior strain. However, replacement would be imminent only in a case where the shared substrate was limiting.

Barros *et al.*, (1984) describe a mixotrophic strain of *T. ferrooxidans* (strain FDI). This strain can utilize both glucose and iron as energy sources, and utilizes both substrates simultaneously. The ability to use glucose may be associated with abilities to use other organic compounds, which may be present in tailings, particularly vegetated tailings, in small quantities. The significance of mixotrophic abilities in avoidance of competition is likely to be confined primarily to the tailings surface where pyrite could become a limiting substrate.

Sugio *et al.*, (1981c) describe two strains of *T. ferrooxidans*, one (AP-19) an obligate iron oxidizer, the other (AP-44) a facultative iron oxidizer with the ability to utilize glucose. Strain AP-44 has the same growth rate in a mixotrophic environment as strain AP-19 on iron, but achieves a higher maximum density based on a greater supply of useable substrate. Thus, it would enjoy a competitive advantage in mixed culture under substrate-limited conditions.

Organic compounds normally inhibit *T. ferrooxidans* activity. Several authors suggest that the apparent close association between *T. ferrooxidans* and various heterotrophs, such as *Thiobacillus acidophilus* and L het 2 (Harrison *et al.*, 1980) or *Acidophilum cryptum* (Harrison, 1981), is a mutualistic association, the heterotrophs utilizing *T. ferrooxidans* waste and preventing organic inhibition in the process. Thus, it is possible that the mixotrophic or facultative heterotrophic strains of *T. ferrooxidans* would interact mutualistically with the wild type. It is also possible that they may have arisen by genetic exchange with associated heterotrophs. Lobos *et al.*, (1984) describe a plasmid which is common to *T. ferrooxidans* and an associated heterotroph (TFC), while Slater and Godwin (1980) emphasize the potential for evolutionary impact of plasmid exchange in interactive microbial communities.

If trophic mutants of *T. ferrooxidans* do interact mutualistically with wild type strains, the interactions would likely be limited to high density conditions when significant concentrations of organic waste are generated, or to surface layers of vegetated tailings where decomposition of plant material occurs.

TABLE 4.2: TROPHIC STRAINS IN THE GENUS THIOBACILLUS

Species (Strains)	Trophic Characteristics	Reference
<u>T. ferrooxidans</u> (FD1)	mixotrophic	Barros <u>et al.</u> , 1984
<u>T. ferrooxidans</u> (AP-44)	facultative iron-oxidizers	Sugio <u>et al.</u> , 1981a
<u>T. ferrooxidans</u> (AP-19)	obligate iron-oxidizers	Sugio <u>et al.</u> , 1981a
<u>T. novellus</u> (?)	amino acid auxotrophy	Davidson <u>et al.</u> , 1985

4.3 Metal Resistance

Much of the strain variation reported in thiobacilli relates to metal ion tolerance. The variability in metal resistance may be a function of its genetic basis, since resistance genes tend to be carried on plasmids which are highly mobile between species and thus have access to a vast gene pool. Some of the resistance factors expressed in thiobacilli are listed in Table 4.3.

Few efforts have been made to demonstrate the plasmid mediation of metal resistant strains in thiobacilli, although the correlation has been well documented in other bacteria (Gardner and Snustad, 1981; Campbell, 1981). Hurtado and Bauer (1985) were unable to identify a specific plasmid carrier of Ag or As resistance in 8 strains of T. ferrooxidans, although plasmids were present in all strains. Martin et al., (1981) identified a plasmid carrier of U-resistance which was present in two U-resistant strains and absent in 13 other non-resistant strains. One of the resistant strains lost its resistance when it lost the plasmid. Eleven of the 15 strains carried plasmid DNA.

The levels of metal tolerance reported in thiobacilli greatly exceed the concentrations that would be expected in uranium tailings porewater. For example, the following porewater concentrations are among the highest reported:

<u>Metal</u>	<u>mg/L</u>
Aluminum	600
Copper	10
Cobalt	8
Uranium	6
Zinc	10
Nickel	10

However, it is possible that much higher concentrations might arise in certain microbial microhabitats, such as in surface tailings exposed to evaporation or during freezing or thawing of interstitial water. Metal resistant strains might enjoy a selective advantage under such temporary adverse conditions, or there may be other metabolic characteristics linked to metal resistance that confer a more general advantage. The

TABLE 4.3:

METAL RESISTANT STRAINS IN THE GENUS THIOBACILLUS

Species (Strains)	Metal	Tolerance	Reference
<u>T. ferrooxidans</u> (M-8/M-8-1)	Cu Zn Ni Co Mo	30/25 ?/g 12/10 ?/g 10/10 ?/g 8/5 ?/g 0.50/0.04 ?/g	Groudev, 1980
<u>T. ferrooxidans</u>	Ag Ag	200 mg/L -	Sugio <u>et al.</u> , 1981 Hurtado and Bauer, 1985
<u>T. ferrooxidans</u>	Hg	1 mg/L	Brierley, 1978
<u>T. versutus</u>	Hg	50 uM	Davidson <u>et al.</u> , 1985
<u>T. versutus</u>	As	100 mM	Davidson <u>et al.</u> , 1985
<u>T. ferrooxidans</u>	As As	50-100 mg/L -	Brierley, 1978 Hurtado and Bauer, 1985
<u>T. ferrooxidans</u>	U (uranyl) U	0.5-1 ₂ g/L ¹	Dispirito and Tuovinen 1981, 1982; Martin <u>et al.</u> , 1981
<u>T. ferrooxidans</u>	Th	0.5-1 g/L	Dispirito and Tuovinen, 1982
<u>T. ferrooxidans</u>	Cu, Al, Co Mn, Ni	10-30 g/L	Lundgren and Dean, 1979; Lundgren and Silver 1980
<u>T. ferrooxidans</u>	Mo Cr	2 g/L 5 g/L	Lundgren and Silver 1980
<u>T. ferrooxidans</u>	Zn	70-120 g/L	Torma <u>et al.</u> , 1970, 1972
<u>T. ferrooxidans</u>	Se, Te	50-100 mg/L	Brierley, 1978

¹ U resistance correlated with high O₂ uptake.² U resistance correlated with a plasmid.

? Units not specified in paper.

high oxygen uptake associated with uranium resistance in T. ferrooxidans (Dispirito and Tuovinen, 1981, 1982) might be one such advantage.

SECTION 5

TIMEFRAMES FOR ADAPTIVE CHANGES

5.0 ESTIMATION OF TIMEFRAMES FOR ADAPTIVE CHANGES

The probability of a geochemically significant adaptive change occurring in a tailings microbial population is a function of the probability of a significant metabolic trait arising in the population, and the ability of that trait to persist. A new trait may arise by mutation within a resident microbial strain, by genetic transfer from a non-resident microbe in a contiguous environment, or by transport of a new microbial strain from a distant population. The persistence of a new trait depends on the advantage which it confers on the host organism in competition with other resident organisms. Degrees of advantage and disadvantage may change with seasonal or long-term changes in the tailings environment.

Neutral mutations, conferring neither a strong advantage nor a strong disadvantage, may persist for long periods at low density and accumulate in a population, thus forming a pool of genetic variation. Some of these rare traits may become advantageous following an environmental change, which thus triggers an adaptive shift.

Microbial transport from a distant population is a complex and highly site-specific phenomenon. Barnthouse and Palumbo (1985) have reviewed microbial transport mechanisms and models. Bovallius *et al.*, (1980) conclude that the best existing models are incapable of predicting microbial transport beyond a distance of about 10 km. Bacteria which remain airborne over larger distances can gain access to the jet stream where Gaussian plume dynamics no longer apply. Bovallius *et al.*, (1978) report long-range bacterial transport from the Black Sea to Sweden, a distance of approximately 2,000 km. Other microbial transport mechanisms include transport by means of surface water, groundwater and animal vectors.

A critical factor in microbial transport is survival in the transport medium. Survival times are often expressed in terms of a decimal reduction time (DRT) representing the time required to kill 90% of the travelling bacterial population. However, the time to kill 100% is probably more relevant to colonization from distant populations, since only a single bacterium is required to establish a new colony. Transport need only be considered over distances that can be travelled within the survival period. Bacteria which form resistant spores under adverse conditions have an effectively infinite survival time; however, thiobacilli do not form spores.

Long-range transport probabilities are not considered further in the discussion that follows, since generic values would not be appropriate, and since their magnitude is probably not sufficient to influence the internal dynamics of an evolving microbial system. Their primary importance would be in redefining the system by introduction of a novel bacterial strain.

The processes of mutation, genetic transfer and strain persistence based on competitive abilities have been incorporated into various population genetic models designed to simulate evolutionary events. A modelling approach to adaptation of microbial populations in tailings is developed in the following sections. A general review of population genetic models is included in Section 5.1. A simple two-strain model for simulating adaptive changes in tailings is proposed in Section 5.2. Its limitations are discussed in Section 5.3. Model parameters are estimated in Section 5.4, based on laboratory and field observations of potentially adaptive bacterial strains. Timeframes for certain specific adaptive changes are estimated in Section 5.5.

5.1 Population Genetic Models

The genetic component of a population genetic model reflects the specific genetic basis of a particular organism characteristic. The characteristic may be determined by a single gene, or by the combined action of multiple genes. In the latter case, the characteristic is said to be 'polygenic' and is typically 'quantitative'. Quantitative traits are continuously distributed in the population. Characteristics determined by a single gene are usually 'qualitative' since they produce a limited number of discrete types in the population. Single gene traits become quantitative when the number of 'alleles' or possible gene states is large.

Examples of evolutionary models for quantitative characteristics are given by Slatkin and Lande (1976), Lande (1976) and Cavalli-Sforza and Feldman (1976). The model of Slatkin and Lande (1976) is strictly phenotypic. A normal distribution of expressed types in the population is assumed, and no specific genetic mechanisms are postulated. Lande's (1976) model, on the other hand, is based on a flexible genetic model with multiple genes. When many genes are involved, phenotypic distributions tend to be normal, as assumed by Slatkin and Lande (1976); however, under strong selection, or with few genes, non-normal distributions can arise. All three models are designed for sexually reproducing

populations, with genetic recombination between parental types at each mating, although similar models for asexual populations could be developed.

Evolutionary models for qualitative characteristics are reviewed by Crow and Kimura (1970). The natural selective pressures which drive the evolutionary process may act either independently of population density, or in a density-dependent fashion. Crow and Kimura (1970) give examples of both types of model. Natural selection is probably density-dependent to some extent in any population that is limited by available food, space or substrate. Under these conditions, there is competition among organisms for limited resources, and strains which use those resources most efficiently enjoy a selective advantage over other strains.

In an unlimited environment, or in the early stages of population growth, the strain with the highest population growth rate enjoys a selective advantage. However, the strain with the highest growth rate is not necessarily the strain which uses its resources most efficiently. Thus, one strain can be favoured by natural selection at low population densities, and a different strain can be favoured under high density conditions. Evolutionary theory denotes the first strain as 'r-selected' and the second strain as 'k-selected', in reference to the logistic growth model parameters r (growth rate) and k (carrying capacity) which dominate under low and high density conditions, respectively.

The logistic growth model in differential form is as follows:

$$\frac{dn}{dt} = rn \frac{(k-n)}{k} \quad (1)$$

where: dn/dt = the instantaneous rate of change in n ,
 n = the population size,
 r = the 'intrinsic growth rate', and
 k = the environmental carrying capacity.

The population stops growing when $n=k$.

A two-strain evolutionary system can be defined by a set of two differential equations in the form of Equation 1, for Strains 1 and 2 respectively:

$$\begin{aligned}\frac{dn_1}{dt} &= r_1 n_1 \frac{(k_1 - n_1 - n_2)}{k_1} \\ \frac{dn_2}{dt} &= r_2 n_2 \frac{(k_2 - n_2 - n_1)}{k_2}\end{aligned}\tag{2}$$

Each strain is characterized by its own growth model parameters, r reflecting its ability to grow quickly at low density, and k reflecting its efficiency at high density. Efficiency is measured in terms of the population size (k) that could be supported by available resources, if each strain occurred alone.

In the system defined by Equation 2, the strain with the highest value of k eventually replaces the other strain, although the rate at which this occurs depends on the relative values of r_1 and r_2 . If $k_1 = k_2$, the strain with the highest value of r eventually replaces the other strain. The selective advantages of Strain 1 can then be defined as follows (Kimura and Ohta, 1971):

$$s = \frac{d}{dt} \ln \frac{p_1}{p_2} = r_1 - r_2\tag{3}$$

where: p_1 = the proportion of the population belonging to Strain 1, and
 p_2 = the proportion belonging to Strain 2.

Under these conditions ($k_1 = k_2$), selection is thus independent of population density (n).

Similar models of population growth based on chemostat theory can be used to define evolutionary systems. The chemostat model takes the following form (SENES, 1986):

$$\frac{dX_p}{dt} = X_p U_{\max} \frac{S}{k_m + S} = \frac{dN_p}{dt} \frac{Y_{x/s}}{A} = -R Y_{x/s}\tag{4}$$

where: X_p = g bacterial biomass/m² of pyrite surface,
 $U_{\max}$ = intrinsic growth rate,

- S = substrate concentration
 k_m = substrate concentration supporting a growth rate of $U_{\max}/2$,
 N_p = mol pyrite,
 $Y_{x/s}$ = growth yield coefficient (g bacterial biomass produced/mol pyrite oxidized),
 A = pyrite surface area (m^2), and
 R = reaction rate of pyrite oxidation.

If several substrates or nutrients are limiting (e.g., P, CO_2 , O_2) the term in S is replaced by a serial product of several such terms. Usually one will dominate. Pyrite itself is apparently seldom limiting, except perhaps in the top millimeter of the tailings pile, since complete pyrite surface area coverage is seldom observed (SENES, 1986). However, irregular pyrite grain geometry and/or jarosite coating of certain microsites may make part of the grain unuseable, leading to more general pyrite limitation. All terms are defined here in relation to pyrite oxidation in tailings, although the model takes the same form for other bacterial cultures.

The chemostat model of population growth is similar to the logistic model of Equation 1 in that initial exponential growth is eventually limited. The main differences are in the expression of population size (mass vs numbers) and the form of limitation. In Equation 4, a stable population size is only achieved when limiting resources are exhausted, leading to extinction. Bacterial washout balancing resource input (e.g., P in rainfall) or mobilization (e.g., pyrite dissolution) must be added to produce a stable finite population. Population size units can be interconverted based on an estimated microbial mass of $2.46E^{-13}$ g wet weight (Baily and Ollis, 1986).

Bacterial washout opens the system, and may be linked to fluxes of limiting nutrients in and out of the tailings (e.g., with rainfall). In the logistic model, these complex kinetics are subsumed in the empirical carrying capacity term.

The logistic model is developed further in Section 5.2 for simulation of adaptive changes in tailings. Model parameters are estimated to characterize particular bacterial strains, and to simulate selection of one strain over the other.

5.2 A Proposed Model

The evolutionary model represented by Equation 2 assumes that Strains 1 and 2 are competing for the same limited resources. However, two strains may differ to some extent in their utilization of substrates, energy sources or carbon sources. This gives the weaker strain at least a partial refuge from the more competitive strain, and may permit co-existence of two strains when one is utilizing different resources than the other. This concept can be incorporated into the two-strain evolutionary system as follows:

$$\begin{aligned}\frac{dn_1}{dt} &= r_1 n_1 \frac{(k_1 - n_1 - \alpha n_2)}{k_1} \\ \frac{dn_2}{dt} &= r_2 n_2 \frac{(k_2 - n_2 - \alpha n_1)}{k_1}\end{aligned}\tag{5}$$

where: α = a competition coefficient representing resource overlap.

Two strains which compete for the same set of resources entirely (i.e., complete resource overlap) are jointly characterized by a competition coefficient of $\alpha = 1$. Under these conditions, Equation 5 reduces to Equation 2.

Levins (1968) discusses estimation of α in terms of resource overlap. A good approximation is as follows:

$$\alpha_{ij} = \frac{\sum_h P_{ih} P_{jh}}{\sum_h P_{ih}^2} = B_i \sum_h P_{ih} P_{jh}\tag{6}$$

where: α_{ij} = the resource overlap between strains i and j,
 P_{ih} = the proportion of strain i utilizing resource h,
 P_{jh} = the proportion of strain j utilizing resource h,
 B_i = the ecological niche breadth of strain i.

Only limiting resources, for which there could be competition, should be included in these calculations.

For simplicity, competition coefficients can be assumed symmetrical (i.e., $\alpha_{12} = \alpha_{21} = \alpha$). This is equivalent to assuming that both strains utilize a resource spectrum of similar breadth, although some of the resources may differ.

Reibesell (1974) illustrates the generalization of the competitive system in Equation 5 to include more than two strains. For simplicity, only two strains are considered here. When two strains are present in the system, the conditions for their stable co-existence, and their equilibrium densities if they coexist, are as follows:

$$\begin{aligned} n_1^* &= \frac{k_1 - k_2}{1 - \alpha^2}, \text{ and} \\ n_2^* &= \frac{k_2 - k_1}{1 - \alpha^2}, \\ \text{if } \frac{k_2}{\alpha} > k_1 \text{ and } \frac{k_1}{\alpha} > k_2, \end{aligned} \tag{7}$$

where: n_1^* = the equilibrium density of Strain 1, and
 n_2^* = the equilibrium density of Strain 2.

Similar conditions and equilibrium densities can be defined for a multi-strain system, although the conditions and calculations are more complex.

The system defined by Equation 5 can be modified to incorporate gene flow between strains. Levin (1978) illustrates the modelling of gene transfer between bacterial strains as a function of their relative densities, using an unlimited population growth model. With limited population growth, as per Equation 5, gene transfer can be incorporated as follows:

$$\begin{aligned} \frac{dn_1}{dt} &= r_1 n_1 \frac{(k_1 - n_1 - \alpha n_2)}{k_1} - \alpha \gamma \frac{n_1 n_2}{n} \\ \frac{dn_2}{dt} &= r_2 n_2 \frac{(k_2 - n_2 - \alpha n_1)}{k_2} + \alpha \gamma \frac{n_1 n_2}{n} \end{aligned} \tag{8}$$

where: γ = the rate of plasmid transfer from Strain 2 (a donor) to Strain 1 (a recipient), and

$$n = n_1 + n_2.$$

This formulation assumes that genetic transfer is essentially one-way, as would be appropriate if Strain 2 was plasmid mediated. In other words, the Strain 2 characteristics are carried on the plasmid. If a copy of the plasmid is transferred to a Strain 1 organism, that organism is converted to Strain 2. The two organisms must come into physical contact to effect such a transfer. Campbell (1981) reviews the role of plasmids in bacterial evolution.

If both strains are plasmid-mediated, the system contains three bacterial types (Strain 1, Strain 2 and Strain 1,2), and gene transfer dynamics are more complex. Plasmid incompatibility systems (Datta, 1979) may select against the recombinant Strain 1,2. This complex situation is not considered.

Gene transfer can also occur by chromosomal conjugation. This too could give rise to a system with three bacterial types. Rates of chromosomal transconjugation are typically much smaller than plasmid transfer rates, and are ignored in the present formulation.

Mutation is another mechanism by which bacterial strains could be interconverted. However, since mutation rates are typically much smaller than plasmid transfer rates, they are unlikely to significantly alter the dynamics of a plasmid-mediated system. Moreover, since plasmids are typically carried in multiple copies, mutational reversion of a plasmid is not likely to modify bacterial performance.

Mutation may be a significant factor in the absence of plasmid mediation. Its main role would be to prevent complete extinction of a strain which is at a selective disadvantage. This effect can be incorporated into the competitive system of Equation 5, as follows:

$$\begin{aligned} \frac{dn_1}{dt} &= r_1 n_1 \frac{(k_1 - n_1 - \alpha n_2)}{k_1} - u n_1 + u n_2 \\ \frac{dn_2}{dt} &= r_2 n_2 \frac{(k_2 - n_2 - \alpha n_1)}{k_2} + u n_1 - u n_2 \end{aligned} \quad (9)$$

where: u = the specific locus mutation rate.

Forward and reverse mutation rates (Strain 1 to Strain 2, and Strain 2 to Strain 1) are assumed here to be equal, and mutations creating new strains are ignored.

Kimura and Otah (1971) illustrate the use of this mutational model in an evolutionary system based on limited population growth with $\alpha = 1$ and $k_1 = k_2$. In a system where Strain 1 is competitively inferior and would otherwise be eliminated, its equilibrium population size is equal to u . In a bacterial species capable of sporulation, complete extinction is highly unlikely in any case, and the role of recurrent mutation in preventing extinction can be effectively ignored.

A computer code incorporating Equations 8 and 9 is listed in Appendix 2. Model limitations, application to the present study, and estimates of evolutionary timeframes based on model simulations are discussed in subsequent sections.

5.3 Model Limitations

The adaptive systems represented by equations (8 and 9) are highly simplified. The combined effects of multiple processes are incorporated into a relatively small number of model parameters. We consider this simplified approach appropriate to the limited quantity and high variability of available data on ecological characteristics of different bacterial strains. Specific characteristics of different strains can ultimately be related, at least qualitatively, to one of the three parameters of the logistic model: growth potential (r), efficiency of utilizing limited resources (k), or types of resources (substrates) utilized in relation to other strains (α). Of these three parameters, k and α are the most important determinants of strain co-existence, while r dictates the timeframe of adaptive change.

The environment is represented as a single, well-mixed aqueous phase, although in fact it is a two-phase solid-aqueous system. Myerson and Kline (1983) have developed a model for bacterial adsorption to solid particles. Their experiments with coal particles in 9K medium without iron suggest that a high proportion (71-100%) of the T. ferrooxidans bacterial population is adsorbed at equilibrium, the proportion decreasing with population density. These experiments span a very short time frame (30 min.), and it is possible that more bacteria exist in the aqueous phase once sufficient iron is released into solution.

However, the entire population certainly does not exist in the aqueous phase, as assumed, and population mixing is therefore over-estimated in equations (8 and 9).

Microsite effects in the solid phase could contribute to lower growth rates in the field, than under laboratory conditions. These effects could lead to localized substrate limitation or growth inhibition at certain sites on the pyrite grain.

The population carrying capacity of the environment is assumed to be constant for each bacterial strain. This implies that the influx of limiting resources (e.g., oxygen) is constant. Equations (8 and 9) also assume that physical-chemical conditions influencing the intrinsic rate of population increase (e.g., temperature, pH) are constant. In fact, both temperature and oxygen influx may vary seasonally. Seasonal effects could be incorporated into the model in the form of algebraic functions for seasonal variation in r and k , or by means of 'look-up' tables for these parameters.

Spatial variation in environmental conditions is also ignored. Temperature, pH and oxygen influx all vary with depth in the tailings. These factors will influence population and evolutionary dynamics. For example, higher temperatures near the surface during summer months will accelerate population growth, along with any adaptive changes that may be taking place in the microbial community.

Model parameter values can be chosen from within the known or expected ranges over the year, or over the depth profile of the tailings, but equations (8 and 9) are probably not sufficient to simulate the net effect of adaptive changes taking place at different rates in different spatial compartments. Simultaneous simulations in several vertically stratified compartments (e.g., surface, unsaturated and saturated zones) with vertical bacterial transport from upper to lower zones might provide a more realistic representation of the adaptive process for the tailings mass as a whole.

The approach taken in subsequent sections is to simulate the horizontal progression of a potential adaptive change, using constant parameter values selected with reference to conditions at a specific depth level. Bacterial interchange between depth levels is not considered.

The horizontal spread of an adaptive change is not incorporated explicitly into the models represented by equations (8 and 9). However, if a constant rate of horizontal

transport is assumed, time and horizontal distance scales can be intercalibrated to estimate the time required for an adaptive change to spread a certain distance from its point of origin.

Genetic drift, or chance variation in gene frequency unrelated to selection pressures on competing strains, is ignored. Genetic drift is a potentially significant evolutionary factor only in very small populations.

Environmental conditions, as represented by model parameters, are assumed to be constant over the time scale of the simulation. Thus, for example, if oxygen influx limits the bacterial carrying capacity of the environment, this influx is constant over the period of adaptive change. Similarly, if nutrients limit the carrying capacity, fluctuating input and nutrient washout are ignored. This does not preclude examination of adaptive response to a postulated environmental change, but, for simplicity, random environmental fluctuations are ignored in the sections that follow.

Random environmental fluctuation could be imposed on the evolutionary system, using Monte Carlo techniques, and statistical distributions for each of the model parameters. However, available data on ecological characteristics of bacterial strains are probably not sufficient to justify this degree of modelling sophistication. Levins (1968) examines the theoretical aspects of evolution in fluctuating environments, and provides a mathematical foundation for the empirical observation that generalist species or strains seem to enjoy a selective advantage in fluctuating environments, while specialists are favoured in a constant environment. Generalist strains are those with broad ecological niches, as per equation (6).

Slater and Godwin (1980) illustrate the principle of selection for the generalist in fluctuating environments, with reference to competing thiobacilli. Thiobacillus neopolitanus, an obligate chemolithotroph, competes successfully with the facultative strain Thiobacillus A2 in chemolithotrophic culture, but is replaced by the facultative strain under mixotrophic chemostat conditions. These conditions constitute both spatial and temporal fluctuation in the environment.

In addition to favouring generalist strains, fluctuating environments can permit coexistence of several strains that otherwise would not coexist. This can occur if the

environment fluctuates between conditions favouring one strain and conditions favouring a different strain, more rapidly than one strain can replace the other. Such a system, in effect, never reaches equilibrium. While model parameters could be manipulated in such a way as to simulate this situation, again using Monte Carlo techniques, the mechanism for doing so is not explicitly incorporated into the adaptive system represented by equations (8 and 9).

5.4 Model Application

Representative ranges for model parameters r , k , γ and u were defined from available literature on behaviour of thiobacilli in laboratory and field situations. However, most of the relevant literature relates to laboratory experiments. Attempts were made where possible to extrapolate from laboratory to field conditions in estimating parameters appropriate to pyritic tailings environments.

Environmental carrying capacity (k) can be estimated from observed bacterial densities in tailings and related environments. Belly and Brock (1974) estimated the number of thiobacilli in coal refuse (pH 1.4) at about 10^8 bacteria/g. SENES (1986) estimated bacterial coverage of pyrite in Denison uranium tailings at 4%, and derived bacterial densities on the order of 10^{10} to 10^{12} bacteria/m² of pyrite, based on pyrite surface coverage and typical bacterial dimensions (SENES, 1985). In tailings with a 1% pyrite content, this is equivalent to about 6×10^7 to 6×10^9 bacteria/g. Brierley (1982) estimated a bacterial density of approximately 10^6 bacteria/g in acid leach liquor. Concentrations in solids may be somewhat higher. The highest concentrations of iron-oxidizers in the Lacnor tailings were on the order of 10^6 bacteria/g (Golder Associates, 1986). From these data, the bacterial carrying capacity of tailings was estimated at approximately 10^8 (10^6 to 10^{10}) bacteria/g for simulations of adaptive changes.

As mentioned in Section 5.3, carrying capacity is likely to be a function of depth in tailings, limited by oxygen influx at most depths.

Intrinsic rate of population increase can be estimated from observed population growth in the exponential phase. Laboratory data often includes the exponential phase, whereas it may be difficult to observe this growth phase in natural tailings. Sources of laboratory data on growth of thiobacilli under optimum or near optimum conditions in synthetic

media are listed in Table 5.1, along with estimates of the intrinsic growth rate (r) derived from these data. Growth rates can alternatively be expressed in terms of the average generation time (T), equal to the population doubling time in a haploid population. The relationship between r and T during exponential growth is as follows:

$$T = \frac{\ln 2}{\ln e^r} = \frac{\ln 2}{r} \quad (10)$$

Both r and T are listed in Table 5.1.

The range in r , based on these data, is approximately two orders of magnitude (0.03 to 0.3 hrs^{-1}). The experimental conditions vary, but are likely more favourable than conditions in most tailings environments. SENES (1986) present an equation for $U_{\max}$ in terms of temperature and pH, based on laboratory studies conducted under natural or semi-natural culture conditions. Based on this equation:

$$r = \frac{13 e^{42.64 - 12883/T_k}}{1 + 10^{2.5 - \text{pH}} + 10^{\text{pH} - 4}} \quad (11)$$

where: T_k = Kelvin temperature, and
 r = intrinsic growth (mth^{-1}).

Assuming a pH range of 1.5 to 6.5 and a temperature range of 270 to 300 °K, r might range from about 10^{-5} to 10^{-2} hrs^{-1} . These growth rates are more consistent with observed rates of pyrite oxidation in the field, and thus may be more representative of growth potential in tailings.

Gene transfer rates, based on laboratory studies with genetic markers on plasmids, are summarized in Table 5.2. These rates may be influenced by many factors, and hence are highly variable. Most studies of plasmid transfer involve plasmids which are not native to thiobacilli. Often the plasmid originates in *E. coli* or *P. aeruginosa*, species which have been better characterized genetically than *T. ferrooxidans*. Well characterized plasmids are more easily manipulated as genetic vectors in strain improvement programs, even though native plasmids are known to occur in most *T. ferrooxidans* strains (Martin et al., 1981).

TABLE 5.1: INTRINSIC GROWTH RATES OF THIOBACILLI

Species (Strain)	Generation Time (T) (hrs)	Intrinsic Growth (r) (hrs ⁻¹)	Reference
<u>T. thioparus</u>	3.3	0.210	Stark and Yanofsky, 1981
<u>T. novellus</u>	8.0	0.087	Perez and Matin, 1980
	11.2	0.062	
<u>T. intermedius</u>	25.0	0.028	Harrison, 1983
<u>T. perometabolis</u>	16.7	0.042	Harrison, 1983
<u>T. ferrooxidans</u>	2.1	0.325	Sugio <u>et al.</u> , 1980
<u>T. ferrooxidans</u> (M8)	6.4	0.108	Groudev, 1980
<u>T. ferrooxidans</u> (M8-1)	4.5	0.155	Groudev, 1980
<u>T. ferrooxidans</u> (NCIB 9490)	6.5-10	0.067-0.107	McGoran <u>et al.</u> , 1969
<u>T. ferrooxidans</u> (NCIB 9490)	14-17	0.041-0.050	McGoran <u>et al.</u> , 1969
<u>T. albertis</u>	13.1	0.053	Bryant <u>et al.</u> , 1983

TABLE 5.2: PLASMID TRANSFER RATES IN THIOBACILLI

Species (Strain)	Plasmid		Transconjugants per Donor-Recipient	Reference
	Origin	Gene	Donor/Recipient	
<u>T. novellus</u>	<u>E. coli</u>	Hg, Te resistance	T.n./T.n.	Davidson <u>et al.</u> , 1985
<u>T. neapolitanus</u>	<u>P. aeruginosa</u> <u>E. coli</u>	resistance	T.n./P.a.	Kulpa <u>et al.</u> , 1983a, b
<u>T. neapolitanus</u>	<u>P. aeruginosa</u> <u>E. coli</u>	resistance	T.n./E.c.	Kulpa <u>et al.</u> , 1983a, b
<u>T. neapolitanus</u>	<u>P. aeruginosa</u> <u>E. coli</u>	resistance	E.c./T.n.	Kulpa <u>et al.</u> , 1983a, b
<u>T. ferrooxidans</u> (FC)	<u>T. ferrooxidans</u> <u>E. coli</u>	origin of replication	T.f./E.c. (RP4) T.f./E.c. (R46) T.f./E.c. (pSa)	10 ⁻² - 2.5x10 ⁻¹ 10 ⁻⁴ - 3.2x10 ⁻⁴ L* 1.5x10 ⁻⁹ Rawlings <u>et al.</u> , 1984
<u>Thiobacillus</u> sp. (A2)	<u>P. aeruginosa</u>	amino acid competence, deficiency	P.a./T.sp. E.c./T.sp. T.sp./E.c. T.sp./T.sp. E.c./E.c.	3.4x10 ⁻⁴ - 10 ⁻³ 8.1x10 ⁻⁴ - 3x10 ⁻³ 6x10 ⁻⁴ - 1.1x10 ⁻³ 10 ⁻² - 1.5x10 ⁻² 1.3x10 ⁻¹ Plasata <u>et al.</u> , 1984

* Less than.

Sanderson (1976) suggests that while chromosomal genes are subject to transfer barriers reflecting phylogenetic relationships between species, genes carried on plasmids may be transferred as easily between as within species. In both cases genetic transfer barriers (or res/mod systems) exist (Roberts, 1976), however plasmid incompatibility systems (Datta, 1979) bear little relationship to bacterial phylogeny. This may be either a cause or a result of the fact that bacterial phylogeny is poorly understood (Stackebrandt and Woese, 1984).

The ease with which plasmids cross strain and species barriers suggests that non-native plasmids may be considered representative of native plasmids in estimation of generic plasmid transfer rates. Indeed the distinctions between native and non-native plasmids may not be particularly useful, especially for plasmids with a broad host range. Available rates of plasmid transfer within and between species display similar ranges of magnitude.

Plasmid transfer rates (γ) between strains and within species of Thiobacillus are represented in Table 5.2 by the work of Davidson et al. (1985) and Plasota et al. (1984). These rates were used for modelling purposes, and range from about 10^{-4} to 10^{-2} transconjugants per donor/recipient pair. The overall range of transfer, including transfer between Thiobacillus and P. aeruginosa and between Thiobacillus and E. coli, is from 10^{-9} to 10^{-1} , depending on the particular plasmids and bacterial strains involved.

Mutation rates (μ) determined in Thiobacillus are listed in Table 5.3. Stark and Yanofsky (1981) and Yanofsky et al., (1983) report mutation rates at amino-acid prototrophy/auxotrophy loci, ranging from about 10^{-9} to 10^{-7} mutations per locus per generation. Davidson et al., (1985) report a reversion frequency for newly acquired As resistance of 1.5×10^{-3} . However, this probably represents the frequency of plasmid rejection since the As resistance factor was plasmid mediated, and the test population consisted of recent transconjugants. The 10^{-9} to 10^{-7} range is typical for bacterial and other populations.

The potential for induction of higher mutation rates as a result of radioactivity in a uranium tailings environment, can be addressed by consideration of typical dose rates to bacteria. Assuming a dose rate in tailings of about 2.3×10^{-4} uGy/h (Section 2.4), an induced mutation rate of 10^{-7} mutations/locus/Gy, and a generation time of 25 h,

TABLE 5.3: MUTATION RATES IN THIOBACILLI

Species	Mutation	Mutation Rate (u)	Reference
<u>T. thioparus</u>	reversion to amino acid prototrophy	5×10^{-8}	Yanofsky <u>et al.</u> , 1983
<u>T. thioparus</u>	amino acid deficiency	10^{-8} - 10^{-9}	Stark and Yanofsky, 1981
<u>T. thioparus</u>	fast growth at low substrate concentration	3.3×10^{-8} - 3.8×10^{-8}	Stark and Yanofsky, 1981
<u>T. versutus</u>	reversion to As sensitivity in recent transconjugants to As resistance	1.5×10^{-3}	Davidson <u>et al.</u> , 1985

5.75×10^{-16} induced mutations/locus/generation can be expected as a result of tailings radiation. This is insignificant relative to the spontaneous mutation rate in bacteria.

Table 5.4 summarizes the range of model parameters used to simulate specific adaptive changes in tailings and estimate their time frames. From equation (11), and typical depth profiles of temperature and pH (Section 3.9), maximum growth potential (r) should occur in the upper tailings during the summer months. Assuming oxygen limitation, the greatest bacterial carrying capacities (k) should also occur near the tailings surface. Actual rates of adaptive change depend on the relative values of r and k in two competing strains (i.e. on the selective advantage enjoyed by the superior strain).

Two possible evolutionary scenarios can be envisaged as follows:

1. a new strain arises de novo, either by introduction from outside the tailings area, or by mutation within the tailings area, or
2. the competitive equilibrium between two co-existing strains is altered as the result of environmental changes favouring one or the other.

With either scenario, one of the strains can be assumed to be plasmid-mediated.

For the first scenario, one set of model parameters is required, including r and k values for each strain. For the second scenario, two such sets of model parameters are required, one before and one after the postulated environmental change. Sets of model parameters for a series of two-strain systems are defined in Tables 5.5. The premise and behavior of each system, and the time required for each system to attain equilibrium, is discussed in detail in Section 5.5.

Intercalibration of time and horizontal distance scales is achieved by assuming a horizontal pore water flow of 10 to 50 m/a. Horizontal flow will be smallest near the surface, where potential growth rates, and adaptive rates (other factors being equal), are high. Horizontal flow will be greatest near the bottom of the active (unsaturated) zone, where growth and adaptive rates are low.

TABLE 5.4: PARAMETER RANGES FOR POPULATION GENETIC MODEL

Parameter	Units	Value	Reference
k	bacteria/g	10^6 - 10^{10}	Brierley, 1982 SENES, 1986
r	hours ⁻¹	0.003-0.3	SENES, 1986 Sugio <u>et al.</u> , 1980
γ	transconjugants/ pair/hr	10^{-4} - 10^{-2}	Davidson <u>et al.</u> , 1985 Plasata <u>et al.</u> , 1984
u	mutations/hr	10^{-9} - 10^{-7}	Stark and Yanofsky, 1981 Yanofsky <u>et al.</u> , 1981

TABLE 5.5: ADAPTIVE CHANGE SCENARIOS (Figures 5.1 to 5.5)

		r_1	k_1	r_2	k_2	α	γ	u
		h^{-1}	bacteria/ g	h^{-1}	bacteria/ g	-	trans/ pair/h	mut/ locus/h
Establishment of a Superior Strain								
Figure 5.1	1B	0.03	1.5×10^8	0.03	0.5×10^8	0.3	0	0
	1C	0.045	10^8	0.015	10^8	0.3	0	0
	2B	0.03	1.5×10^8	0.03	0.5×10^8	1	0	0
	2C	0.045	10^8	0.015	10^8	1	0	0
Figure 5.2	1B	0.03	1.5×10^8	0.03	0.5×10^8	0.3	0	0
	1C	0.045	10^8	0.015	10^8	0.3	0	0
	1K	0.03	1.5×10^8	0.03	0.5×10^8	0.3	0	10^{-7}
	1L	0.045	10^8	0.015	10^8	0.3	0	10^{-7}
Figure 5.3	1B	0.03	1.5×10^8	0.03	0.5×10^8	0.3	0	0
	1C	0.045	10^8	0.015	10^8	0.3	0	0
	1F	0.03	1.5×10^8	0.03	0.5×10^8	0.3	0.01	0
	1G	0.03	0.5×10^8	0.03	1.5×10^8	0.3	0.01	0
	1H	0.045	10^8	0.015	10^8	0.3	0.01	0
	1I	0.015	10^8	0.045	10^8	0.3	0.01	0
Figure 5.5	5H	0.0045	10^6	0.0015	10^6	0.3	0.01	0
	5I	0.015	10^6	0.045	10^6	0.3	0.01	0
	1H	0.045	10^8	0.015	10^8	0.3	0.01	0
	1I	0.015	10^8	0.045	10^8	0.3	0.01	0
	4H	0.45	10^{10}	0.15	10^{10}	0.3	0.01	0
	4I	0.015	10^{10}	0.045	10^{10}	0.3	0.01	0
Figure 5.4	1F	0.03	1.5×10^8	0.03	0.5×10^8	0.3	0.01	0
	1H	0.045	10^8	0.015	10^8	0.3	0.01	0
	1I	0.015	10^8	0.045	10^8	0.3	0.01	0
Adverse Environmental Change								
Figure 5.4	3F	0.03	0.5×10^8	0.03	0.1×10^8	0.3	0.01	0
	3H	0.015	10^8	0.003	10^8	0.3	0.01	0
	3I	0.003	10^8	0.015	10^8	0.3	0.01	0

5.5 Temporal-Spatial Simulation of Adaptive Changes

5.5.1 Resource Overlap

Figure 5.1 illustrates the importance of resource overlap in determining the population genetic consequences of a superior strain introduction. As discussed in Section 4.0, the superior strain may enjoy either an r (intrinsic growth) or k (efficiency during limited growth) advantage. These different types of advantage are represented by the C and B model runs, respectively.

Runs 1B and 1C show that, with a sufficiently small resource overlap (α), the superior and inferior strains can co-exist indefinitely. The critical value of α , at which co-existence becomes possible, depends on the relative values of k_1 and k_2 . The inequality defining this critical value is given in Equation 7. The equilibrium strain densities can be expressed as an algebraic function of the k and α parameters (Equation 7).

Runs 2B and 2C show that, with complete resource overlap, only one strain can persist. This strain is the one with the k advantage or, if the k values are equal, the one with the highest initial density.

5.5.2 Mutation Rate

Figure 5.2 illustrates the negligible effect of mutation rate on the establishment of an introduced superior strain, with either a k advantage (top row) or an r advantage (bottom row). In either case, the course of strain establishment with a high rate of mutation ($u = 10^{-7}$ mutations/locus/hour) is virtually identical to that which occurs in the absence of mutation. Equivalent forward and reverse mutation rates are assumed, as described in Section 4.0.

5.5.3 Plasmid Transfer Rate

Figure 5.3 illustrates the effects of plasmid transfer on establishment of a superior strain, under k advantage (top row) and r advantage (bottom row) scenarios. In either case, the population genetic change without plasmid transfer (left) can be compared to the course of change with either the inferior (centre) or superior strain (right) as the plasmid-mediated (donor) strain. A high rate of plasmid transfer ($\gamma = 0.01$) is assumed.

Runs 1F, 1H and 1I show that the equilibrium densities of two co-existing strains can be influenced by plasmid transfer, which gives the donor strain an added advantage. Run 1G shows that plasmid transfer can also result in extinction of an inferior strain, when the donor strain enjoys a k advantage.

The population genetic significance of plasmid transfer depends on the range of growth model parameters (Section 5.5), as well as the degree of advantage otherwise enjoyed by the superior strain. This is discussed in greater detail in Section 5.5).

5.5.4 Adverse Environmental Changes

Figure 5.4 illustrates some effects of hypothetical environmental changes on co-existence of two bacterial strains. An adverse environmental change is postulated which reduces either the r or k parameter of both competing strains. The previously inferior strain is assumed to be more sensitive to this adverse effect, as reflected in a greater r or k reduction.

The Series 1 model runs (left) depict the establishment of the superior strain following its introduction to the system. After equilibrium is achieved, the environment changes suddenly, altering the equilibrium points. The Series 3 model runs (right) depict the population genetic shift toward the new equilibrium points. In some cases (3F and 3I), the inferior strain is eliminated as an indirect result of the environmental change. In other cases (3H), the two strains continue to co-exist.

Run 3H illustrates the fact that a plasmid-mediated donor strain is more resistant to such adverse environmental changes. In spite of a greater environmental impact on its population growth rate, the donor strain increases slightly, while the recipient strain declines to extinction.

5.5.5 Population Growth Parameter Range

Figure 5.5 illustrates the effect of the r and k range on establishment of a superior strain. At high r and k values (right), equilibrium is achieved more rapidly, and the strains co-exist more equitably than at lower r and k values (centre and left). At the lowest r and k values (left), co-existence may be impossible, and the donor strain enjoys a more definite advantage.

The parameter range, and the r value in particular, dictate the timeframe for an adaptive shift to reach equilibrium. At low r values, timeframes may approach 5,000 h, with strain parameter differences of $\pm 50\%$. At high r values, timeframes may be as short as 120 h. Time to equilibrium increases as the strain differences (i.e., relative advantages) decrease; however, so does the significance of an adaptive shift from one strain to the other.

5.5.6 Timeframe and Spatial Progression of Adaptive Shifts

Figure 5.6 illustrates the spatial progression of the slowest (Figure 5.6A) and fastest (Figure 5.6B) adaptive shifts obtained, within the range of generic model parameters considered reasonable for tailings microbial populations. Horizontal movement is assumed to occur at either 10 m/a or 50 m/a. Based on these rates, time and distance scales can be intercalibrated. The adaptive shift is nearest completion at its spatial point of origin, and further from completion at greater distances from this point.

Timeframes for completion of an adaptive shift at its spatial point of origin range from 120 h (Figure 5.6B) to 5,000 h (Figure 5.6A). Timeframes for completion at 2 m from the point of origin range from 480 to 7,000 h. Thus, within a year, adaptive shifts on the order of $\pm 50\%$ r or k , originating at a single point, could be completed over a radius of approximately 2 to 48 m. This estimate is contingent on the model assumptions listed in Section 5.3 and on the range of model parameter values, particularly r values, input to the model.

The time to acidification of a pyritic tailings mass is on the order of two to three years (Belly and Brock, 1974). Thus, rapid adaptive changes could theoretically affect significant areas prior to acidification, and potentially influence the acidification process. However, any such influence depends on the introduction of a geochemically significant strain, by in situ mutation, or transport from beyond the tailings. The probabilities of such introductions cannot be assessed from the scanty empirical data available on specific bacterial strains in tailings environments.

FIGURE 5.1: EFFECTS OF COMPETITION (α) ON ESTABLISHMENT OF A NEW SUPERIOR STRAIN

IB and 2B: Strain I k Advantage
 IC and 2C: Strain I r Advantage

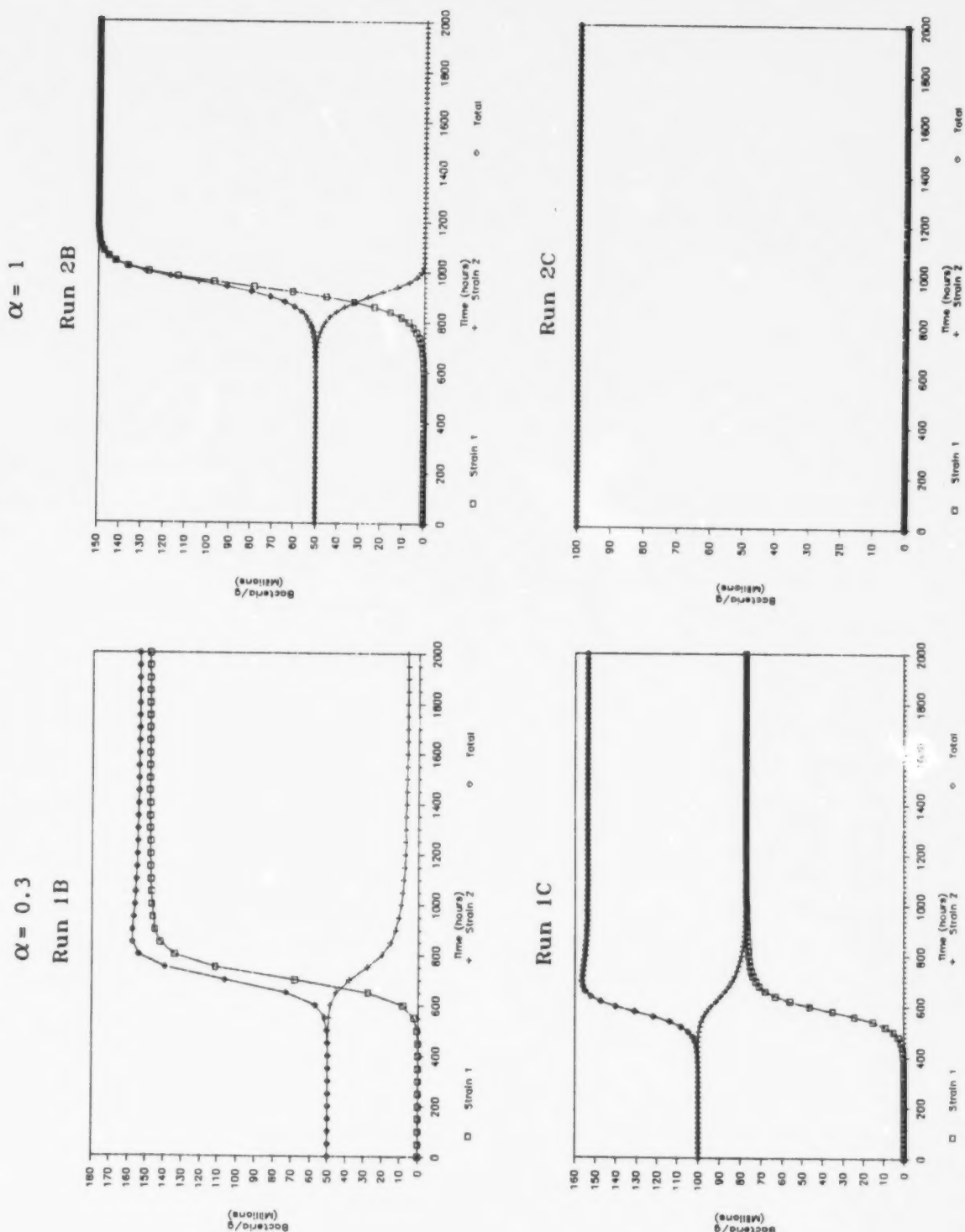


FIGURE 5.2: EFFECTS OF MUTATION (u) ON ESTABLISHMENT OF A NEW SUPERIOR STRAIN

IB and IK: Strain I k Advantage
 IC and IL: Strain I r Advantage

$$u = 10^{-7}$$

$$u = 0$$

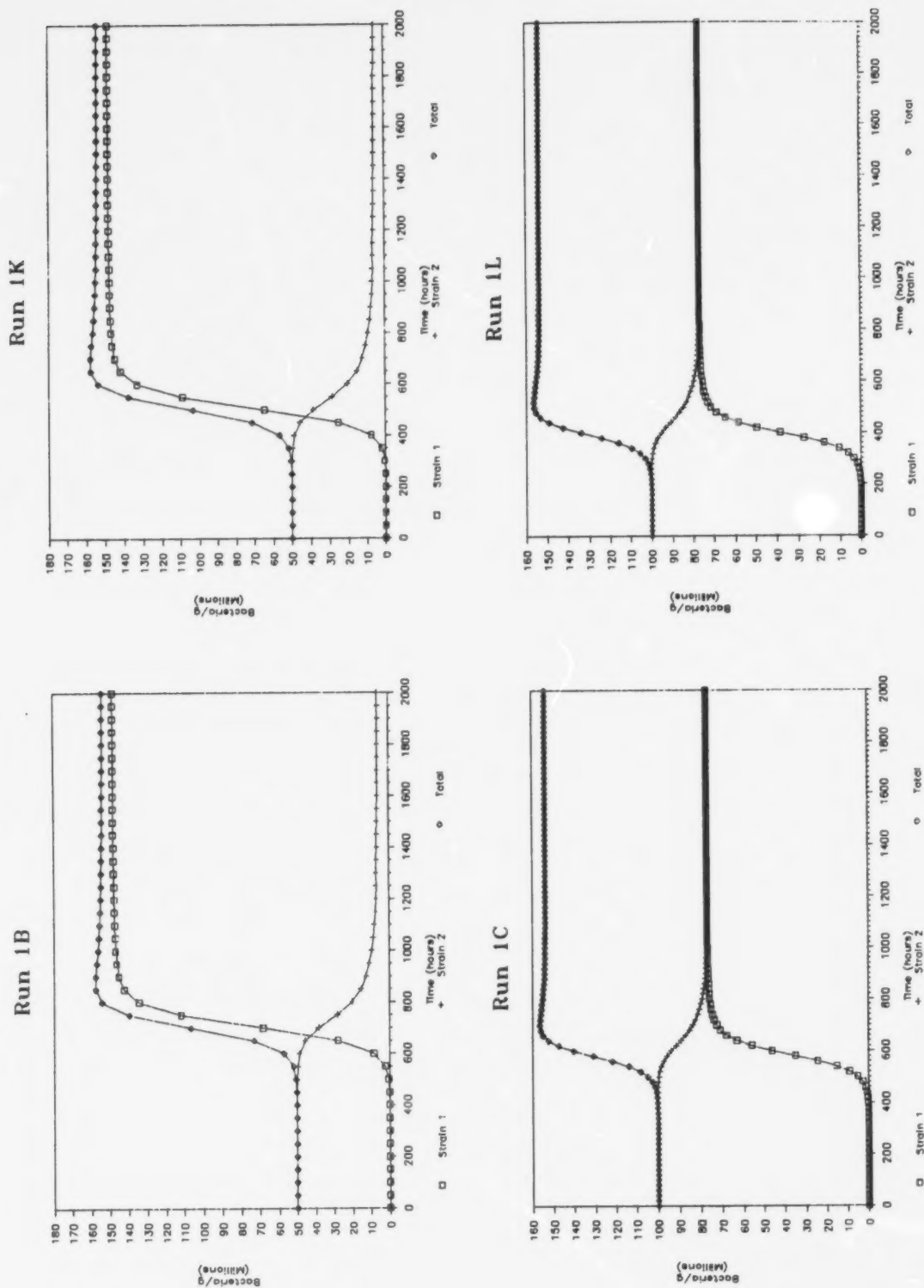


FIGURE 5.3: EFFECTS OF PLASMID TRANSFER (γ) ON ESTABLISHMENT OF A NEW SUPERIOR STRAIN (Plasmid Mediation of Strain 2)

IB and IF: Strain 1 k Advantage
 IC and IH: Strain 1 r Advantage

IG: Strain 2 k Advantage
 II: Strain 2 r Advantage

$\gamma = 0$

$\gamma = 0.01$

$\gamma = 0.01$

Run 1B

Run 1F

Run 1G

Run 1C

Run 1H

Run 1I

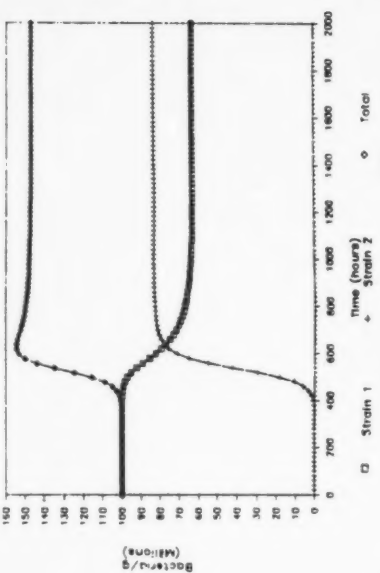
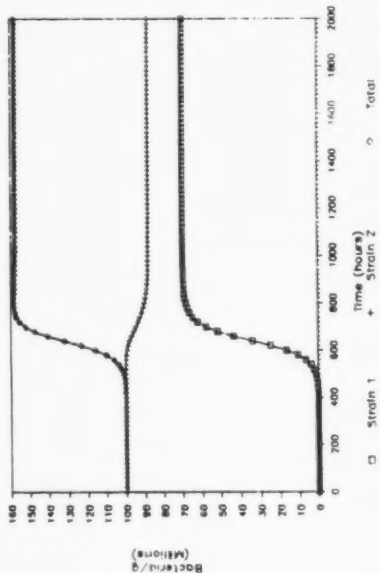
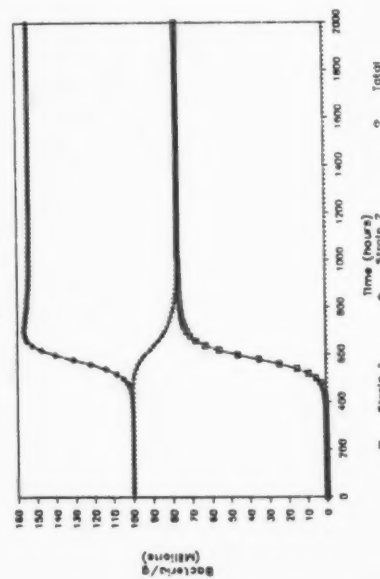
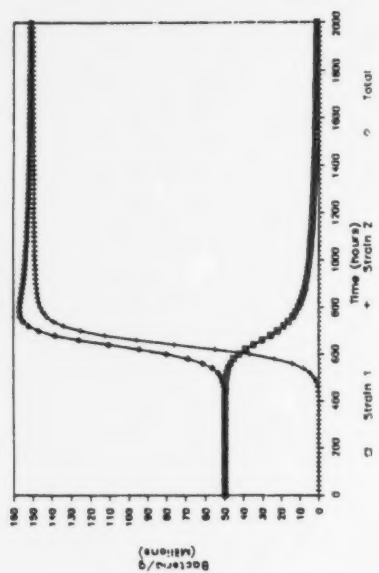
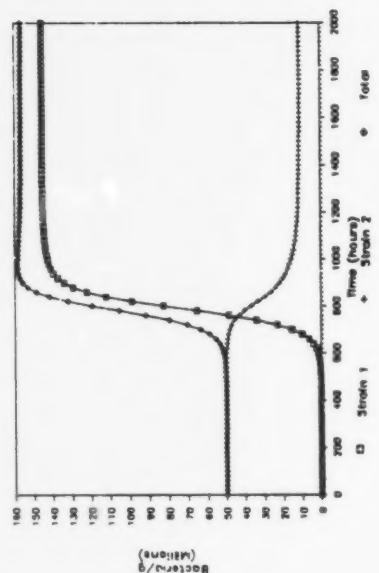
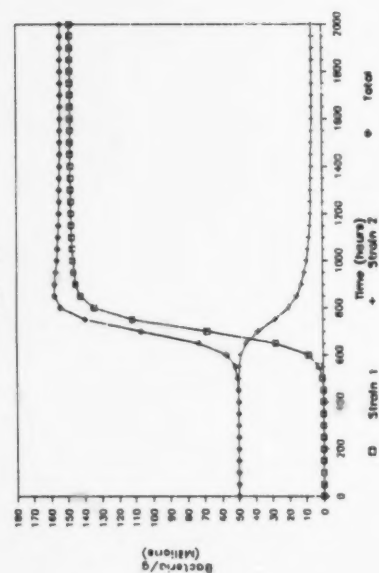


FIGURE 5.4: EFFECTS OF INCREASED ENVIRONMENTAL SEVERITY ON COEXISTING STRAINS, WITH GREATEST IMPACT ON THE INFERIOR STRAIN (Plasmid Mediation of Strain 2, $\gamma = 0.01$)

1F-1I: Establishment of Superior Strain
3F-3I: Response to Environmental Change

F: Strain 1 k Advantage
H: Strain 1 r Advantage
I: Strain 2 r Advantage

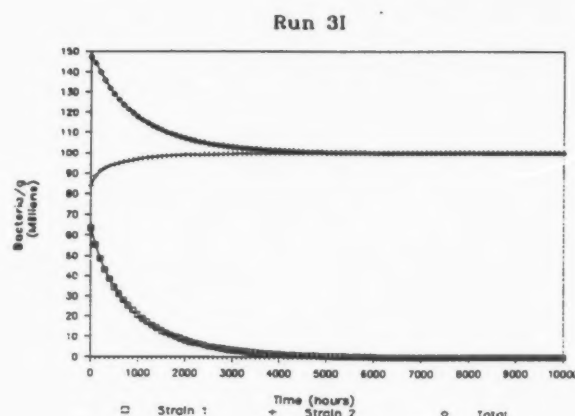
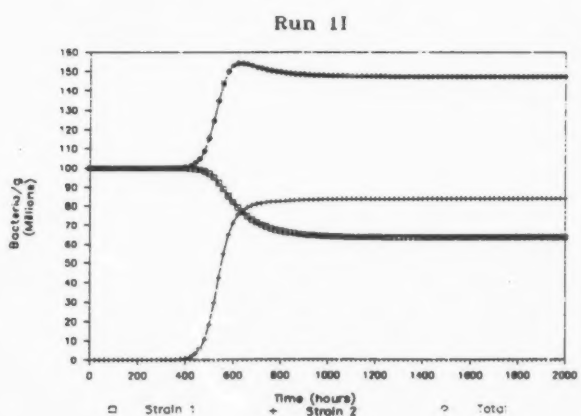
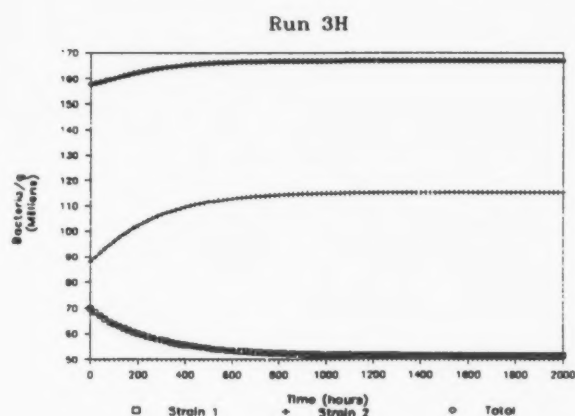
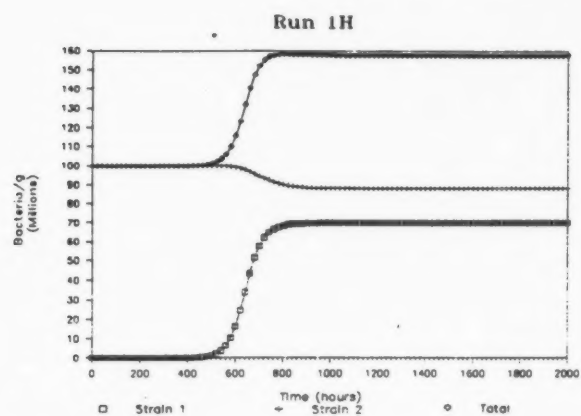
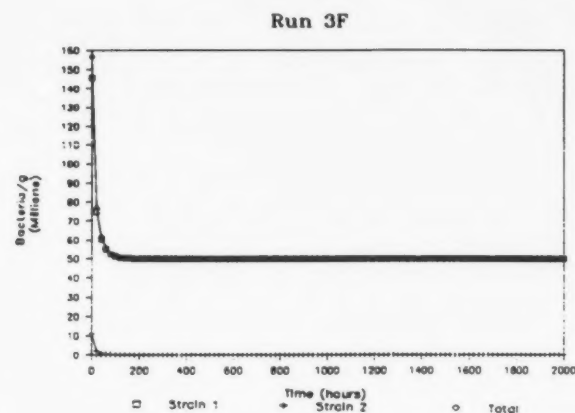
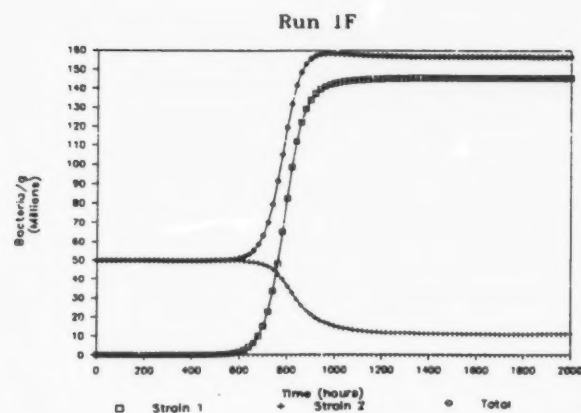


FIGURE 5.5: EFFECTS OF POPULATION GROWTH MODEL PARAMETER RANGE ON ESTABLISHMENT OF A NEW SUPERIOR STRAIN (Plasmid Mediation of Strain 2, $\gamma = 0.01$)

H: Strain 1 r Advantage
I: Strain 2 r Advantage

Median
Parameter
Values:
 $k: 10^6/g^{-1}h^{-1}$
 $r: 0.003 h^{-1}$

$k: 10^8/g^{-1}h^{-1}$
 $r: 0.03 h^{-1}$

$k: 10^{10}/g^{-1}h^{-1}$
 $r: 0.3 h^{-1}$

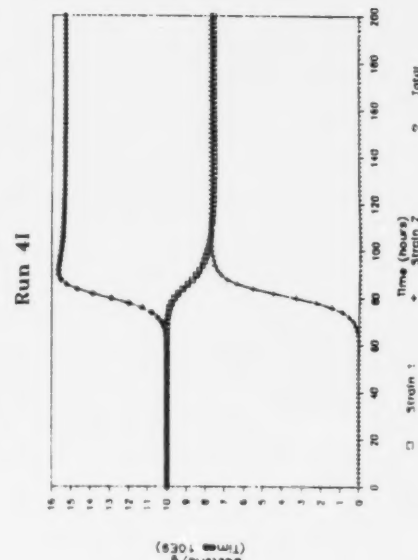
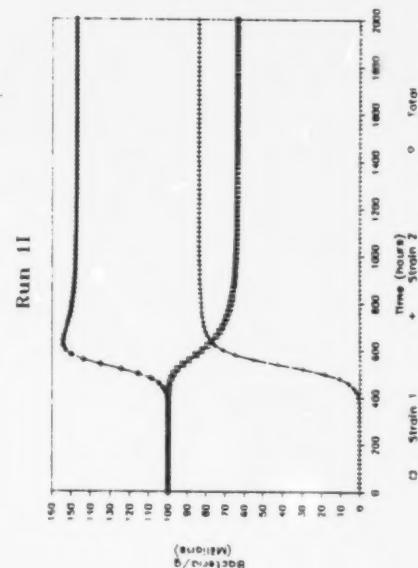
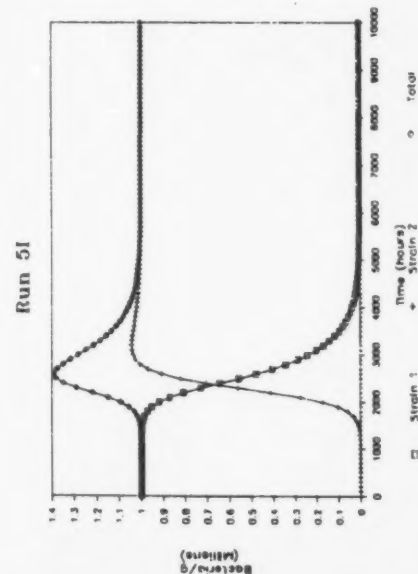
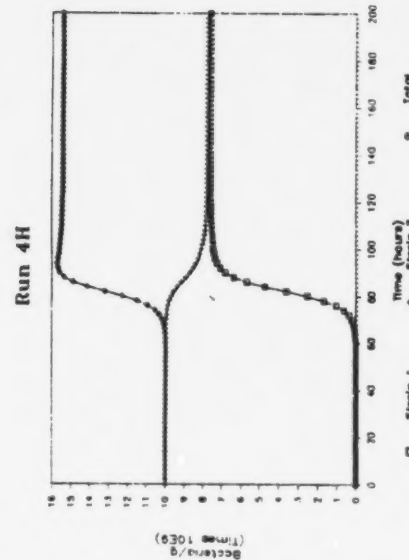
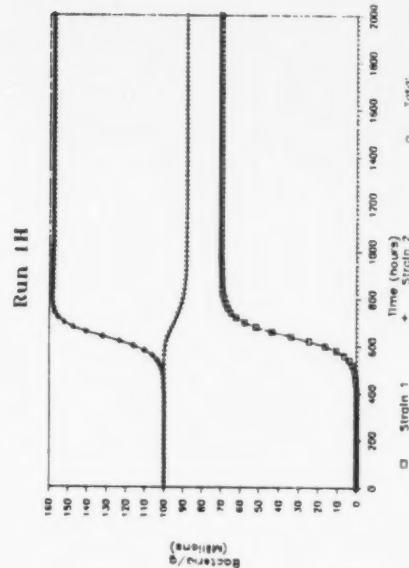
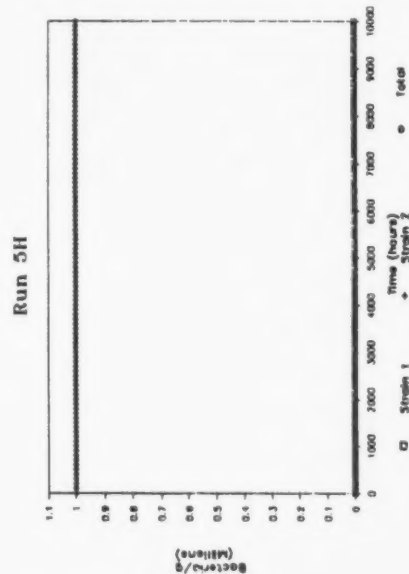
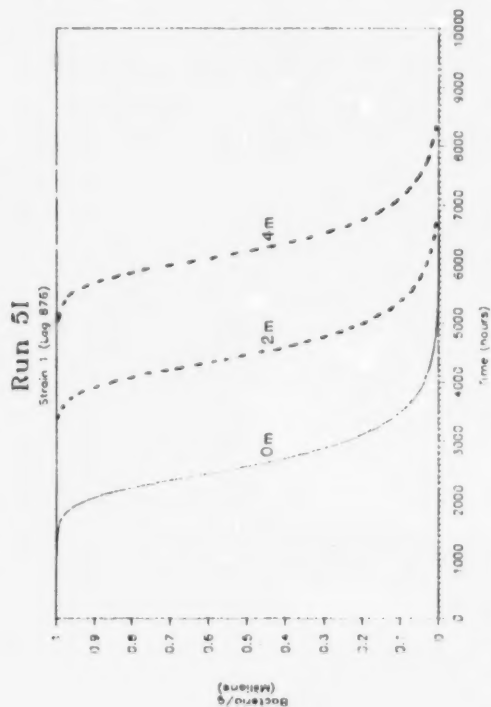


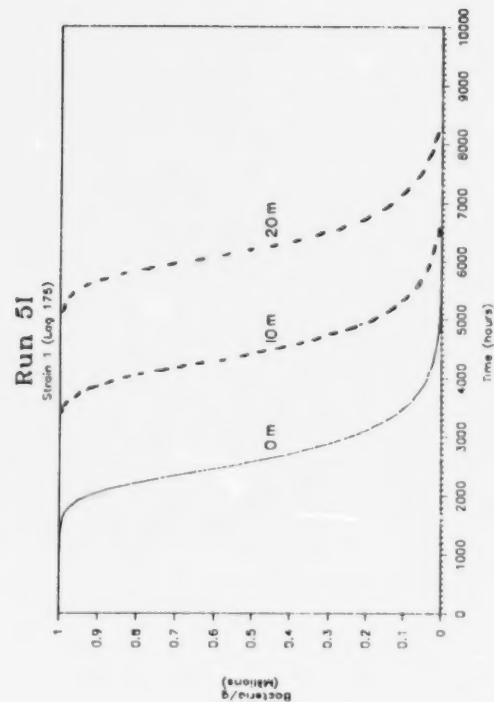
FIGURE 5.6A: SPATIAL PROGRESSION OF ADAPTIVE SHIFTS FROM POINT OF ORIGIN
(Strain 2 Establishment with r Advantage, Plasmid Mediation, $\gamma = 0.01$, $k = 10^{10}/g$, Median $r = 0.3 \text{ h}^{-1}$)

Horizontal
Movement:

10 m/a



50 m/a



Run 5I
Strain 2 (Log 876)

Bacteria/g (Millions)

Time (hours)

Run 5I
Strain 2 (Log 175)

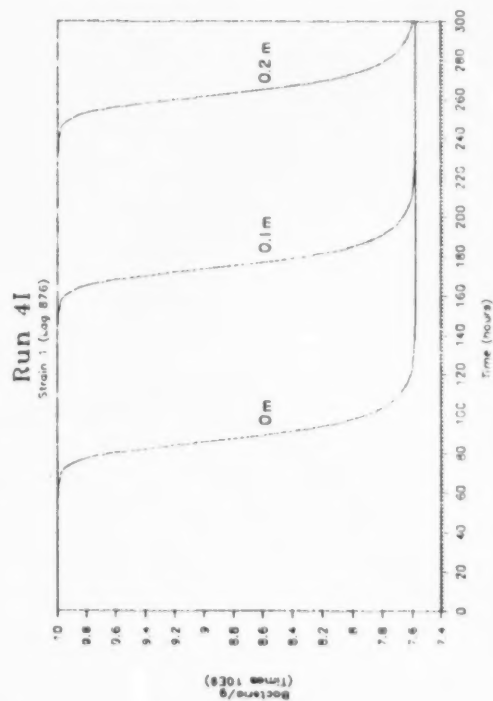
Bacteria/g (Millions)

Time (hours)

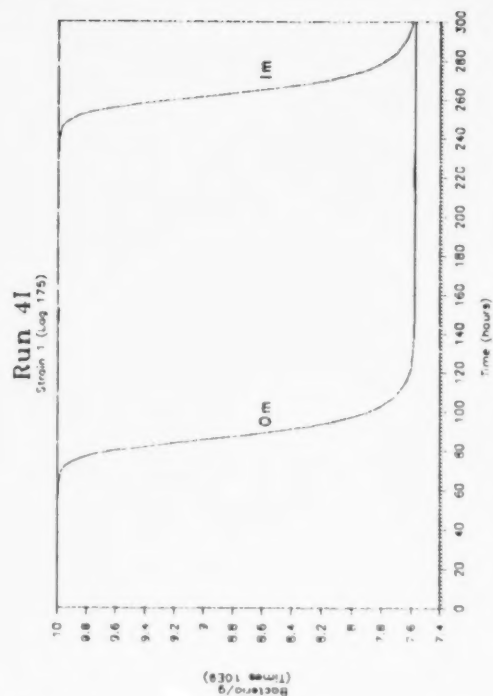
FIGURE 5.6B: SPATIAL PROGRESSION OF ADAPTIVE SHIFTS FROM POINT OF ORIGIN
(Strain 2 Establishment with r Advantage, $\gamma = 0.01$, $k = 10^6/\text{g}$, Median $r = 0.003 \text{ h}^{-1}$)

Horizontal
Movement:

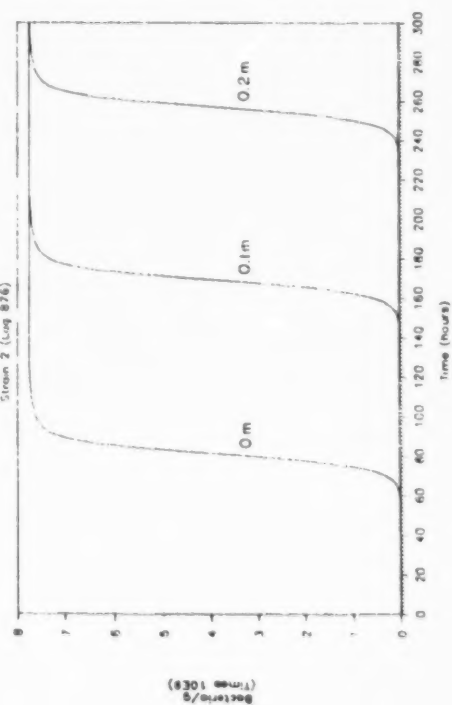
10 m/a



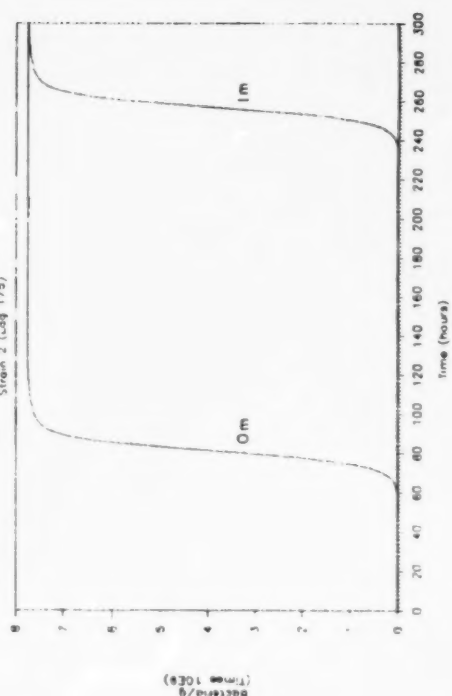
50 m/a



Run 4I
Strain 2 (Log 876)



Run 4I
Strain 2 (Log 175)



SECTION 6

CONCLUSIONS AND RECOMMENDATIONS

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Review of the literature on different strains of thiobacilli, studied primarily in the laboratory, indicates strain variation in several categories that could potentially influence the process of uranium tailings acidification, mineral dissolution and radionuclide release. The most important strain characteristics in this context involve either oxidation rates or substrate utilization. Metal resistance characteristics are highly variable, and highly mobile within and between species, but are probably of little geochemical consequence except when linked genetically to oxidation capabilities.
2. The logistic growth model provides an adequate population genetic framework within which to consider evolutionary consequences of new strain introduction, although model refinements to incorporate effects of bacterial adsorption, and random and seasonal environmental fluctuation, will be required for improved simulation of adaptive changes. In terms of logistic model parameters, oxidation rates influence the intrinsic growth rate (r), and substrate utilization influences carrying capacity (k) and resource overlap between strains (α).
3. The greatest obstacle to accurate prediction of adaptive shifts in tailings is a paucity of empirical data on bacterial species and strains in uranium tailings environments, strain differences between localities, and in situ rates of growth, genetic exchange and movement. Rates of aerial dispersal and survival during long-range transport are critical to estimation of strain introduction probabilities.
4. Microcosm studies of specific strain population dynamics and strain interactions would help to refine population genetic models and model parameters. These studies involve simulation of tailings environments in the laboratory, and could make effective use of genetic probes for strain identification.
5. Estimated timeframes of adaptive change, given introduction of a new stain, and using generic model parameters over a wide range, suggest that introduced

strains can become rapidly established in tailings populations. Establishment over a significant area is estimated to occur within a year. This period is short relative to geochemical timeframes in tailings (e.g., two to three years for acidification) and thus adaptive changes could theoretically influence geochemical processes if geochemically significant strains were introduced.

6. Bacterial mutation rates do not seem sufficient to significantly alter the process of strain establishment or the timeframes involved. However, mutation may be very important in generating new strains and maintaining population variability.
7. Plasmid transfer rates reported for thiobacilli and other bacteria are highly variable. The highest rates reported for thiobacilli are sufficient to alter both the timeframes of strain establishment, and the equilibrium densities established.

6.2 Recommendations

1. An analysis of the species, strains and total populations present in representative mine tailings in an attempt to verify the proposed population genetic model. This would entail starting with fresh tailings and following the colonization process, as well as looking at older tailings to assess the populations present. Both thiobacilli and heterotrophs should be enumerated. Both viable counts and direct microscopic counts should be done. The viable counts may be difficult, but at least they would give some idea of the Thiobacillus population in the tailings. A reliable agar plate technique for viable counts of T. ferrooxidans has recently been developed (B. Butler, University of Waterloo, pers. comm.). The direct counts would provide some idea of total biomass present.
2. Experimental verification of the proposed model. This could be attempted by introducing a "marked" Thiobacillus culture into the tailings. Antibiotic resistance would be a good marker since carriers could be re-isolated and counted. If the relative growth rates of the indigenous bacteria were known and the introduced strain growth rate was slightly different, it might be possible to follow subsequent competitive interactions in the field. Even if this could not be done because of technical problems, it still might be done in a laboratory experiment (e.g., Yates et al., 1986).

3. Some estimate of the "biologically-available carbon" in the system. This could be obtained quite simply in a "biological oxygen demand" type of experiment: sterilize the tailings, add heterotrophic bacterial culture and measure oxygen uptake and population increases. This would give some idea of the amount of potential biomass due to heterotrophic growth and might be useful in assessing the extent of autotrophic growth.
4. Examination of the effects of aging on the tailings. This could involve sampling different tailings of known ages and histories. An analysis of the sulphur moieties present, the thiobacilli and the heterotrophs present, and the physical properties of different tailings might give some useful information.
5. Examination of the effects of water cover on the overall tailings acidification process. This might provide a control measure to reduce leaching, or it might lead to other microbial processes becoming predominant (e.g., Fedorak *et al.*, 1986). The experiment would be relatively simple, although it might take a long time.

7.0 BIBLIOGRAPHY

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APPENDIX 1

Glossary

APPENDIX 1: GLOSSARY

Acidophiles - organisms which live at low pH:

- o Facultative Acidophiles: organisms which tolerate high concentrations of H^+ ions.
- o Obligate Acidophiles: organisms which require high concentrations of H^+ ions (possibly because H^+ ions may be required for the membrane stability of these organisms).

Aerobes:

- o Facultative Aerobes: organisms which are able to use alternative electron acceptors when oxygen is not available and do not require oxygen for biosynthesis.
- o Obligate Aerobes: organisms which require oxygen for growth and biosynthesis (i.e., cannot use alternative electron acceptors).

Anaerobes - organisms which are unable to use oxygen as the terminal electron acceptor

- o Aerotolerant Anaerobes: organisms which do not use oxygen, but are not harmed by it and thus are able to grow in the presence or absence of oxygen.
- o Obligate Anaerobes: organisms which do not use oxygen and to which oxygen is toxic.

Autotrophs - organisms which obtain all their cellular carbon by the fixation of CO_2 using the Calvin cycle, which is unique to them for CO_2 reduction:

- o Chemoautotrophs: organisms which obtain their cellular carbon from CO_2 and obtain all their energy requirements by the oxidation of inorganic substrates.
- o Facultative Autotrophs: organisms which use CO_2 as the carbon source and organic compounds as the energy source.

Auxotrophs - mutant organisms lacking the capacity of forming an enzyme present in the parental strain and, therefore, requiring a supplementary substance for growth.

Bacteriophage - virus which uses bacterial hosts.

Carrying Capacity - the maximum population size (per unit area or volume) that can be sustained by available resources (energy supply, nutrients, space, etc.).

Chemolithotrophs - organisms obtaining nourishment from inorganic mineral sources.

Cryptic Genes - sequences for coding characteristics which are no longer important or used, but are maintained in the population. The selective advantage appears to be to allow rapid population response to new conditions without hindering survival under current situations.

Ecological Niche Breadth - a measure of the range and diversity of resources (e.g., substrates) used by a species. A species with a broad niche is considered a generalist, while one with a narrow niche is a specialist.

Finite Growth Rate - the actual rate of population growth under limited conditions.

Heterotrophs - organisms which use organic compounds both for cellular carbon and as an energy source.

Intrinsic Growth Rate (r) - the exponent describing the rate of exponential population growth under unlimited conditions (also potential growth rate or maximum growth rate).

Locus - the location of a single gene on a chromosome or extra chromosomal DNA segment.

Microaerophiles - organisms which require oxygen at pressures lower than that found in air (i.e., lower than 0.2 atmospheres).

Mixotrophs - organisms which use organic compounds as the carbon source and inorganic compounds as the energy source.

Phylogeny - (the study of) evolutionary relationships between organisms. (Ideally, taxonomic systems of classification should reflect these relationships.)

Plasmid - circular piece of DNA which is separate from the bacterial chromosome. These can replicate themselves as well as incorporate and transfer chromosomal DNA:

- o **Conjugative Plasmids:** those which are easily transferred between bacteria.

Procaryotes - cellular microorganisms, consisting of the bacteria and blue-green algae, which have a nuclear region not bounded by a membrane.

Prophage - DNA found in a bacterium and originating from a virus.

Prototrophs - organisms nourished from one type of energy supply or in one manner only.

Psychrophiles - organisms thriving at relatively low temperatures, below 20°C.

Res/Mod System - restriction and modification systems found on plasmids, chromosomes and phages which restrict transfer of genetic material by breaking down foreign DNA.

Thermophile - an organism which has an optimum growth temperature above 45°C.

Transconjugants - organisms which have recently obtained new genetic characteristics by conjugation with another organism.

Transduction - transport of bacterial DNA from one host to another by a virus (bacteriophage).

Transformation - uptake of DNA material from the surrounding medium (e.g., dead sources).

Transposons - genetic elements which promote DNA movements within microbial genomes. They can facilitate the transfer of a gene from a chromosome to a plasmid.

APPENDIX 2

Computer Code of Population Genetic Model

```

10 INPUT "What is the R1 value ":R1
20 INPUT "What is the R2 value ":R2
30 INPUT "What is the K1 value ":K1
40 INPUT "What is the K2 value ":K2
50 INPUT "What is the value ":ALPHA
60 INPUT "What is the value ":GAMMA
70 INPUT "What is the value ":MU
80 INPUT "What is the dt value ":DT
90 INPUT "What is the dc value ":DC
100 INPUT "What is the tmax value ":TMAX
110 INPUT "Input the initial values for n1, n2 ":N1,N20
120 INPUT "Do you want output on a printer. Y/N? ":ASK$
130 INPUT "Do you want output on a file. Y/N? ":ASK2$
140 N1=N10:N2=N20:N=N1+N2
150 PRINT USING "&": " T " P1 " P2 " RBAR " KBAR " N1 " N2
160 PRINT USING "####.### " :T::PRINT USING "###.###" :P1,P2,RBAR,KBAR,N1,N2,N
170 C=0
180 IF (ASK$="Y") OR (ASK2$="Y") THEN GOSUB 490
190 IF (ASK2$="Y") OR (ASK2$="Y") THEN GOSUB 620
200 FOR T=0 TO TMAX STEP DT
210 ALP*=(ALPHA*GAMMA*N1*N2)/N
220 'RINT ALP*
230 IF (ALP* > ALPHA*N1) THEN ALP*=ALPHA*N1
240 'RINT ALP*
250 DN1DT*=(R1*N1)*((K1-N1-ALPHA*N2)/K1)-ALP*+MU*N2-MU*N1
260 DN2DT*=(R2*N2)*((K2-N2-ALPHA*N1)/K2)-ALP*+MU*N2-MU*N1
270 'RINT DN1DT*
280 'RINT DN2DT*
290 'TOP
300 N1OLD=N1:N2OLD=N2:NOLD=N
310 N1=N1OLD-CSNG(DN1DT*)*DT
320 N2=N2OLD-CSNG(DN2DT*)*DT
330 IF N1<1! THEN N1=1
340 IF N2<1! THEN N2=1
350 N=N1+N2
360 P1=N1/N
370 P2=N2/N
380 RBAR=P1*R1-P2*R2
390 KBAR=P1*K1-P2*K2
400 IF C <> DC GOTO 460
410 PRINT USING "####.### " :T::PRINT USING "###.###" :P1,P2,RBAR,KBAR,N1,N2,N
420 IF (ASK$="Y") OR (ASK2$="Y") THEN GOSUB 590
430 IF (ASK2$="Y") OR (ASK2$="Y") THEN PRINT #1,T,N1,N2,N
440 C=0
450 GOTO 460
460 C=C+1
470 NEXT T
480 END
490 REM subroutine to print output on printer
500 WIDTH "LPT1:".160
510 LPRINT CHR$(12)
520 GOSUB 670
530 INPUT "What run title is this? ":TITLES
540 LPRINT USING "&": " TITLES
550 LPRINT " "
560 LPRINT USING "&": " T " P1 " P2 " RBAR " KBAR " N1 " N2
570 LPRINT USING "####.### " :T::LPRINT USING "###.###" :P1,P2,RBAR,KBAR,N1,N2,N
580 RETURN
590 REM subroutine to print rest of output on printer
600 LPRINT USING "####.### " :T::LPRINT USING "###.###" :P1,P2,RBAR,KBAR,N1,N2,N
610 RETURN
620 REM subroutine to print output in a file
630 INPUT "What is the name of the output file? ":OUFIL$
640 OPEN OUFI$ FOR OUTPUT AS #1
650 PRINT #1,T,N1,N2,N
660 RETURN
670 LPRINT " R1 = ":R1
680 LPRINT " R2 = ":R2
690 LPRINT " K1 = ":K1
700 LPRINT " K2 = ":K2
710 LPRINT " Alpha = ":ALPHA
720 LPRINT " Gamma = ":GAMMA
730 LPRINT " Mu = ":MU
740 LPRINT " Dt = ":DT
750 LPRINT " Dc = ":DC
760 LPRINT " Tmax = ":TMAX
770 LPRINT " N1 = ":N1
780 LPRINT " N2 = ":N2
790 LPRINT " N = ":N
800 LPRINT CHR$(12)
810 RETURN

```